ESTIMATION OF ANTI-OXIDANT AND ANTI -DIABETIC EFFECT OF *NITOPHYLLUM MARGINALE* (J.AGARDH) IN ALLOXAN INDUCED DIABETIC RATS

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Abbreviations

N.marginale	*Nitophyllum marginale*
T2DM	Type 2diabetes mellitus
DM	Diabetes mellitus
MBAI	Marine biota in India
DMS:	Degree minutes seconds
CTAB	Cityltrimethylammoniumbromide
PCR	Polymerase chain reaction
rBcl	Ribulose-bisphosphatecarboxylase
GAE	Gallic acid equivalent
LD	Lethal dose
DMSO	Dimethylsulfoxide
DPPH	1,1-diphenyl-2-picryl hydrazyl
ABTS	2,2 –azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)
NO	Nitric oxide
H_2O_2	Hydrogen peroxide
Conc.	Concentration
i.p	Inhibitory percentage
DNSA	Diitro salicylic acid
GOM	Gulf of mannar
OECD	Organization of economic and development
PUFA	Polyunsaturated fatty acids
BMW	Body mass weight
W/V	Weight per volume
V/V	Volume per volume
TBARS	Thiobarbituric reactive acid substances
BSA	Bovine serum albumin
PNPG	4-Nitrophenylβ-D-glucopyranoside
DMEM	Dulbecco's Modified Eagle's Medium
TBARS	Thiobarbituric reactive acid substances
IAEC	Institutional Animal Ethics Committee
H_2O_2	Hydrogen peroxide
LPO	Lipid peroxidation
mM	mile molar
HDL	High density lipid
LDL	Low density lipid
VLDL	Very low density lipid
TP	Total protein
SGOT	Serum Glutamic Oxaloacetic Transaminase
SGPT	Serum Glutamic Pyruvate Transaminase
TGL	Triglycerides
ALP	Alkaline phosphate
HB	Haemoglobin
BGL	Blood glucose level
P.P	Post-prandial
mg/ml	milligram per milliliter
NIPER	National institute of education and research
CMFRI	Central marine fisheries research institute
HPTLC	High performance thin layer chromatography
GC-MS	Gas chromatography/Mass spectrometry
b.w	Body weight
DTNB	5,5-dithio-bis-(2-nitrobenzoic acid)
NCBI	National center for biotechnology information

GLUT	Glucose transporter
LFT	Liver function test
RFT	Renal function test
GR	Glutathione reductase
MDA	Malondialdehyde
CAT	Catalase
O.D	Optical density
AOAC	Association of official Analytical chemists
i.p	Intraperitoneally
ANOVA	Analysis of variance
HPE	Histo pathology examination
DPX	Dibutylphthalate polystyrene xylene
N.S	Normal saline
GOD POD	Glucose oxidase peroxidase
RIA	Radio immuno assay vial
PBS	Phosphate buffer saline
FCR	Folin Ciocalteau's reagent
S.D	Standard deviation
S.E.M	Standard error mean
IU/L:	International unit per liter
mmol/L	Millimolperliter
UV-VIS	Ultraviolet-visible spectrophotometer
Nm	Nanometer
TAC	Total Antioxidant capacity
Conc.	Concentration
Rpm	Revolution per minute
C.F	Centrifugal force
TPC	Total Phenolic content
IU/L:	International unit per liter
TFC	Total Flavonoid content
TAC	Total Alkaloid content
TSC	Total Saponin content
TCC	Total carbohydrate content
CS	Chemical stability
CP	Chemical potential
BC	Bioactive compounds
YE	Yield extract
ROS	Reactive oxygen species

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Abstract

This study examines Type 2 Diabetes Mellitus (T2DM), a complicated metabolic condition characterised by insulin resistance, impaired pancreatic β-cell activity, and changes in glucose homeostasis.The escalating global incidence of T2DM necessitates innovative and comprehensive therapeutic approaches. In recent times, natural products have gained considerable attention due to their diverse bioactive compounds, holding promise for pharmaceutical and nutraceutical development. Among the natural sources, seaweeds have emerged as a valuable reservoir of bioactive molecules with diverse pharmacological properties. In the present research, samples were systematically collected from the Gulf of Mannar, Tamil Nadu, India, in November 2022. Morphological identification using standard taxonomic keys confirmed the sample as *Nitophyllum marginale*. This investigation aims to explore the potential of seaweed-derived bioactive metabolites in addressing the multifaceted aspects of T2DM, contributing to the development of novel therapeutic strategies. The study embark on an extensive investigation in to the phytochemical composition, antioxidant potential, antidiabetic activity and invivo effects of methanolic extract derived from *N.marginale*, a species of red seaweed. The phytochemical profile of *N.marginale* was explored using a combination of qualitative and quantitative analysis, employing two distinct solvents chloroform and methanol. The comprehensive approach enables to elucidate the diverse array of phytoconstituents present in the seaweed extract, providing insights in to its chemical composition. The finding showed the presence of saponins, tannins, terpenoids, steroids, glycosides, flavonoids, alkaloids, quinones, phenols, curcumins and carbohydrates was determined through qualitative analysis using solvents chloroform and methanol. Antioxidant activity was assessed through a battery of assays including 1,1-diphenyl -2-picryl hydrazyl (DPPH) radical scavenging, 2,2 azino bis (3-ethyl benzothiazoline-6-sulfonic acid) (ABTS) radical cation scavenging, lipid peroxidation inhibition and measurement of nitric oxide and hydrogen peroxide scavenging capacities. *N.marginale* extracts in chloroform and methanol demonstrated concentration-dependent increases in DPPH radical inhibition, with methanol extract exhibiting the highest percentage (69.57± 0.52) with respect to chloroform (28.92 ± 0.77). In the ABTS assay, *N.marginale* extracts in methanol and chloroform showed concentration-dependent trends. Methanolic extract displayed superior free radical scavenging activity at (59.93 ± 0.76), surpassing chloroform extract at (38.79 ± 0.74), A graph depicting lipid peroxidation assay results for *N. marginale* extracts at different concentrations showed methanolic extract demonstrated an inhibitory concentration of 69.34

± 0.59) while chloroform exhibited (27.54 ± 0.28) inhibition. At a higher concentration (1.0 mg/ml), the methanolic extract displayed a nitric oxide scavenging activity of (52.58 ± 0.367), whereas the chloroformic extract exhibited a slightly lower percentage at (29.12 ± 0.536) with reference standard (at higher conc. 1 mg/ml as Ascorbic acid) showed inhibition (90.86±0.42).The effectiveness may be attributed to the presence of phenolic compounds, flavonoids or other bioactive components known for their antioxidant properties. These evaluations shed light on the potential of *N.marginale* extract to mitigate oxidative stress, a hallmark of various chronic diseases. The antidiabetic properties of the extract are investigated using in vitro assays such as Alpha amylase inhibition assay, Results indicated the ability of the extracts to inhibit alpha-amylase enzyme responsible for starch breakdown in to simpler sugars. Methanol consistently displayed higher percentage inhibition values of 43.85% while chloroform extract shows 38.66%. Alpha glucosidase inhibition assay shows inhibition rate at higher percentage (1.0 mg/ml) with respect to methanolic extract shows 40.92% with chloroform shows 32.43% and glucose uptake assay with L6 cell line shows inhibition rate with methanol 40.80%and chloroform 34.27% cytotoxicity using 3T3L1 cell line. The invivo efficacy of the methanolic extract is evaluated using an alloxan-induced diabetes model in experimental animals (wistar rats). The high doses of alloxan i.e 150mg/kg were used to induce type 2 diabetes (T2DM) in male wistar rats. The rats with Blood glucose level above 250 mg/dl were considered for study. The rats were divided in to 6 group's i.e GI, GII, GIII, GIV, GV and GVI. This model enables for the examination of many biochemical parameters linked with diabetes, including blood glucose levels, lipid profiles, and indicators of oxidative stress. Histopathological examinations are also conducted to elucidate the impact of the extract on pancreatic morphology and function. This multidimensional study provides a comprehensive understanding of the pharmacological potential of *N.marginale* extract, highlighting its phytochemical diversity, antioxidant capabilities, antidiabetic effects, and invivo efficacy.

The results outlined in this study add to the expanding collection of data endorsing the therapeutic effectiveness of bioactive compounds derived from seaweed, thereby facilitating the exploration of new natural products aimed at addressing diabetes and its related complications. This research contributes to the foundation for the creation of innovative solutions for the management of diabetes and its associated health issues.

Keywords: T2DM, Bioactive compounds, *Nitophyllum marginale*, phytochemicals, anti-oxidants, anti-diabetic, nutraceutical, pharmaceutical.

CHAPTER-1

INTRODUCTION

Summary: This chapter is about the introduction to the Research work. The chapter has been divided into six parts.

1. Introduction

1.1 Diabetes Mellitus

Diabetes mellitus (DM) is a persistent medical condition marked by heightened levels of glucose (sugar) in the blood. This condition develops either because the pancreas produces insufficient insulin or the body fails to effectively utilize the produced insulin. Insulin, a crucial hormone for controlling blood sugar levels, is involved in this process. Diabetes can be caused by genetic factors or lifestyle choices, such as poor diet and lack of physical activity. If left unmanaged, diabetes can lead to serious complications, including vision impairment, kidney disease, and nerve damage(Baynest, 2015),(Chawla, 2013). Effective management involves maintaining a healthy lifestyle, monitoring blood sugar levels, and, in some cases, using medications like insulin (Riaz, 2009). International Diabetes Federation (IDF) revealed the prevalence of T2DM estimated to be 7.5 % (374 million) in 2019 and projected to reach 8.0% (454 million) by 2030 and 8.6%(548 million) by 2045 (Saeedi et. al., 2019), (Jarrar et. al., 2023). Diabetes mellitus comprises various categories, such as Type 1 diabetes, marked by autoimmune β-cell destruction, and Type 2 diabetes, characterized by progressive β-cell insulin secretion loss alongside insulin resistance. Gestational diabetes arises during pregnancy, while other forms result from factors like monogenic syndromes, exocrine pancreas disorders, and drug-induced causes. The American Diabetes Association's classification guides tailored therapies (Alberti & Zimmet, 1998). However, distinctions between Type 1 and Type 2 diabetes at diagnosis are not always clear, challenging the traditional age-based paradigms. Diabetic ketoacidosis may occur in Type 2 diabetes, and while classic symptoms typically manifest in children, adults may exhibit more variable signs, emphasizing the evolving understanding of diabetes types across age groups. Despite initial diagnostic complexities, clarity often emerges over time, emphasizing the importance of flexible approaches in diabetes management (Tests & Diabetes, 2016)

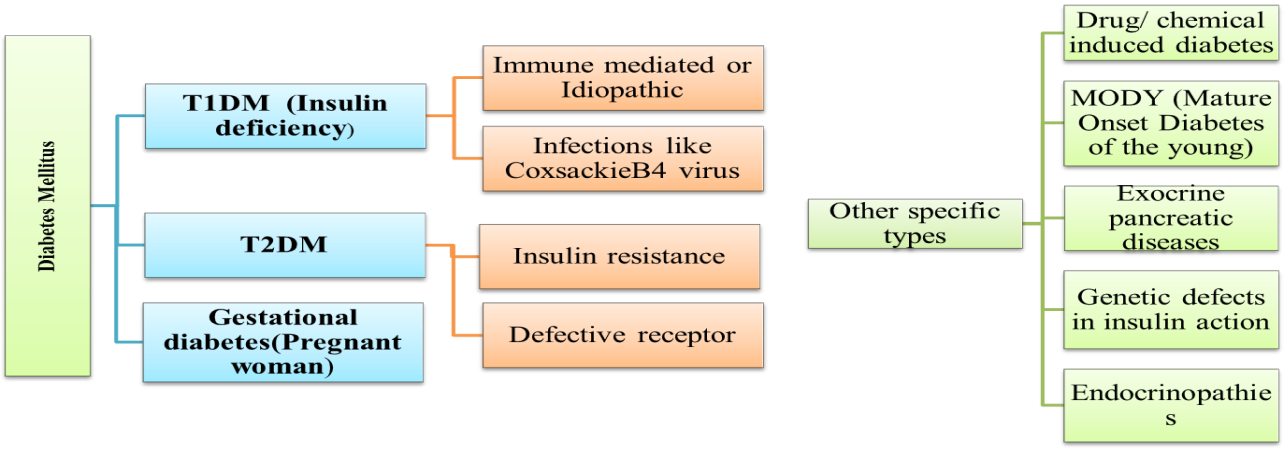

Fig 1: Classification of Diabetes mellitus(Webber, 2013)

1. Type One Diabetes:

Type 1 diabetes develops due to an autoimmune process where pancreatic beta cells are destroyed, leading to inadequate insulin production..Commonly diagnosed in children and young adults, it can occur at any age. Treatment primarily involves insulin therapy, with essential management through regular blood glucose monitoring (David Leslie et al., 2021)

2. Type 2 Diabetes:

Type 2 diabetes, the most prevalent kind, arises when the body becomes resistant to insulin or generates insufficient amounts.Typically diagnosed in adults, it is increasingly prevalent in children and adolescents due to lifestyle factors. Treatment involves lifestyle modifications (diet and exercise), oral medications, and occasionally insulin therapy (Professional Practice Committee: Standards of Medical Care in Diabetes-2020)

3. Gestational Diabetes:

Gestational diabetes develops during pregnancy when the body doesn't produce sufficient insulin to meet the heightened demands, thereby elevating the risk of complications during pregnancy and delivery..Typically emerging during pregnancy, it often resolves after

childbirth. Treatment involves dietary adjustments, exercise, and occasionally insulin therapy(Obstetric Analgesia and Anesthesia, 1996)

4. Monogenic Diabetes:

Monogenic diabetes arises from mutations in a single gene, impacting insulin production or function. Onset can happen at any age, even in infancy. Treatment varies based on the specific genetic mutation, ranging from oral medications to insulin therapy(Rubio-Cabezas et al., 2014)

5. Secondary Diabetes:

Secondary diabetes stems from another medical condition or medication side effects. Onset varies depending on the underlying cause. Treatment involves addressing the root condition and may include insulin therapy or other diabetes medications(Diabetes, 2010)

These classifications help guide healthcare professionals in developing appropriate treatment plans and interventions for individuals with diabetes. It is important to note that diabetes is a complex and multifaceted condition, and individuals may experience variations within each type. Additionally, ongoing research may lead to further refinements in the classification and understanding of diabetes (Mcdermott & Trujillo, 2020)

1.2 Pathophysiology of Type 2 Diabetes Mellitus

Type 2 diabetes mellitus (T2DM) is a multifaceted metabolic disorder characterized by high blood sugar levels (hyperglycemia) resulting from a blend of insulin resistance and diminished insulin secretion.The pathophysiology of T2DM encompasses various elements, encompassing genetics, lifestyle factors, and underlying physiological alteration (Westman, 2021)

- **Insulin Resistance**: Reduced responsiveness to insulin is a prominent characteristic of T2DM. Insulin, a hormone synthesized by the pancreas, plays a crucial role in managing blood sugar levels. In Type 2 Diabetes, the body's cells exhibit decreased sensitivity to insulin, leading to a reduction in the uptake of glucose from the bloodstream. (Matboli et al., 2021). As a result, this leads to higher levels of glucose circulating in the bloodstream, known as hyperglycemia.

- **Beta Cell Dysfunction:** In addition to insulin resistance, Type 2 diabetes often impacts the production of insulin by pancreatic beta cells. Initially, these beta cells compensate for insulin resistance by increasing their insulin secretion. However, with time, these beta cells may become fatigued and unable to release sufficient amounts of insulin, exacerbating hyperglycemia (Rachdaoui, 2020)
- **Glucose Overproduction:** The liver has a notable role in controlling blood sugar levels. In T2DM, the liver might persist in generating surplus glucose, even when elevated levels are already present in the bloodstream. This excessive glucose production by the liver further contributes to hyperglycemia(Ramatchandirin et al., 2023)
- **Incretin Hormone Dysfunction:** Incretin hormones as well as glucagon-like peptide-1 (GLP-1), are released from the gastrointestinal tract in reaction to food consumption. These hormones stimulate insulin secretion, reduce glucagon release (a hormone that increases blood sugar levels), and slow down gastric emptying. In T2DM, there may be a decrease in the release or action of incretin hormones, leading to impaired insulin secretion and increased glucagon levels(Drucker & Holst, 2023)
- **Adipose Tissue Dysfunction:** Fat cells, develop in adipose tissue, play a vital role in the metabolism of sugar as well as contents of elevated level of fats . In individuals with obesity and T2DM, adipose tissue undergoes dysfunction, releasing elevated levels of fatty acids and other substances known as adipokines. These substance can modify the signalling of insulin and helps in the development of insulin resistance(Keshvari et al., 2022)
- **Chronic Low-grade Inflammation:** Inflammation is a hallmark of T2DM. Adipose tissue dysfunction and obesity contribute to the release of pro-inflammatory substances, leading to chronic low-grade inflammation. Inflammatory mediators can impair insulin signaling pathways, exacerbating insulin resistance(Goossens & Blaak, 2015)
- **Genetic Factors:** There is a genetic predisposition to developing T2DM. Certain gene variants can increase the risk of insulin resistance and impaired insulin secretion. However, genetic factors alone are not sufficient to cause

T2DM and are often combined with environmental and lifestyle factors(Piko et al., 2021)

- **Lifestyle Factors:** A lack of physical activity, unhealthy dietary habits (rich in refined carbohydrates and saturated fats), and obesity are key factors that contribute significantly to the onset of T2DM. These elements can exacerbate insulin resistance and elevate the likelihood of developing the condition (Uusitupa, 2002)

The pathophysiology of T2DM is complex and involves a combination of interrelated factors. The understanding of T2DM pathophysiology is still evolving, and ongoing research is focused on elucidating the underlying mechanisms and identifying potential therapeutic targets for effective management and prevention of T2DM.

1.3 Treatment of diabetes by using oral antidiabetic drugs:

These medicines are mostly used to manage type 2 diabetes, where the body either produces insufficient insulin or grows resistant to its effects. These medications help lower blood sugar levels and improve insulin sensitivity (Bailey & Day, 2003). However, it's important to note that type 1 diabetes requires insulin therapy and cannot be managed solely with oral antidiabetic drugs(Alberti & Zimmet, 1998). Here are some common classes of oral antidiabetic medications:

- **Metformin:** Typically, metformin is the initial medication prescribed for type 2 diabetes. Its mechanism involves decreasing the liver's glucose production and enhancing insulin sensitivity throughout the body. Metformin may also help with weight management. Side effects can include gastrointestinal issues like nausea and diarrhoea(Grammatiki et al., 2020)
- **Sulfonylureas:** This category of medications induces the pancreas to increase insulin production. Examples include glipizide, glyburide, and glimepiride. Potential side effects may encompass low blood sugar levels (hypoglycemia), weight gain, and gastrointestinal disturbances(Hossain & Pervin, 2018)
- **Meglitinides:** These medications work similarly to sulfonylureas but have a shorter duration of action. They stimulate insulin release from the pancreas. Repaglinide and nateglinide are commonly used Meglitinides. Side effects can include hypoglycemia and weight gain (Thulé, 2012)

- **Thiazolidinediones (TZDs):** TZDs enhance insulin sensitivity in the body's cells and diminish glucose production by the liver. Rosiglitazone and pioglitazone serve as examples of TZDs. Potential side effects comprise weight gain, fluid retention, and an elevated risk of fractures (Gastaldelli et al., 2006)
- **Dipeptidyl Peptidase-4 (DPP-4) Inhibitors:** It helps to lower blood sugar levels by increasing insulin production while lowering glucose synthesis in the liver. Some DPP-4 inhibitors that are often prescribed are sitagliptin, saxagliptin, and linagliptin. Side effects are generally modest and can include upper respiratory tract infections and headaches (X. Wang et al., 2018)
- **Sodium-Glucose Cotransporter-2 (SGLT2) Inhibitors:** It work by reducing glucose reabsorption in the kidneys, which leads to increased glucose excretion through the urine. This group of drugs can also help with mild weight loss and blood pressure control. Canagliflozin, dapagliflozin, and empagliflozin are all examples of SGLT2 inhibitors. Increased urination, urinary tract infections, and an increased risk of genital fungal infections are some of the potential side effects.(Ni et al., 2020)

Individual patient features, medical history, renal function, and overall treatment goals all play a role in selecting oral antidiabetic medication(s) (Chaudhury et al., 2017). The prescribing healthcare professional will determine the most suitable medication and dosage for each individual. Regular monitoring of blood sugar levels and close follow-up with a healthcare provider are essential for successful management of diabetes.

1.4 Need of Alternative medication like seaweeds for diabetes:

Seaweeds have gained popularity as an alternative to traditional treatment for a variety of reasons. First of all, they are heavily packed with vital minerals such as iodine, calcium, magnesium, iron, and vitamins C and K (Choudhary et al., 2021). These nutrients are crucial for various bodily functions and can help address deficiencies when incorporated into the diet (A. K. Pandey et al., 2020).Moreover, seaweeds contain several bioactive compounds, including polysaccharides, peptides, phlorotannins, and polyphenols (Gani et al., 2016). These compounds have showed a wide range of medicinal activities, including anti-inflammatory, antioxidant, antibacterial, antiviral, anticoagulant, and anticancer actions.(Meinita et al., 2022). As a result, seaweeds have been used in traditional medical systems for ages to treat a variety of illnesses. Research has also highlighted the potential health advantages connected with seaweed eating.(Pérez-Lloréns et al., 2023). For instance,

seaweeds may support thyroid function due to their iodine content, improve cardiovascular health by reducing cholesterol levels and blood pressure, enhance immune function, promote gut health through prebiotic effects, and aid in weight management by promoting satiety and reducing fat absorption (Smyth, 2021). Furthermore, seaweeds provide a sustainable and environmentally beneficial alternative to traditional medicine.They are abundant in the world's oceans and can be harvested without causing significant harm to marine ecosystems. This stands in contrast to many conventional medicines, which often rely on synthetic compounds derived from finite resources or plants that require extensive land and water resources for cultivation(Sudarwati et al., 2020).Additionally, the cultural and traditional use of seaweeds in coastal communities around the world has contributed to their recognition as a valuable source of food and medicine. Traditional knowledge passed down through generations has long recognized the health benefits of seaweeds, further fueling their exploration in modern healthcare (Mokhena et al., 2016). Overall, seaweeds hold promise as a natural and sustainable alternative to conventional medicine due to their nutrient density, bioactive compounds, potential health benefits, sustainability, and cultural significance (Thurstan et al., 2018). While further research is needed to fully understand their mechanisms of action and therapeutic potential, seaweeds offer a compelling avenue for promoting health and wellness in both traditional and modern contexts.

1.5 Importance of seaweeds:

Seaweeds, also known as macroalgae, play a crucial role in marine ecosystems, and they have various applications with economic, environmental, and health-related significance. Here are some key aspects highlighting the importance of seaweeds and their applications:

- **Biodiversity and Ecosystem Health:**
  Seaweeds contribute to maritime biodiversity by providing habitat and food for a wide range of marine creatures. They support the health and balance of coastal habitats (Palumbi et al., 2009)
- **Nutrient Cycling:**

Seaweeds play a role in nutrient cycling by absorbing nutrients from the water. This not only supports their own growth but also helps regulate nutrient levels in coastal areas (Cotas et al., 2023)

- **Aquaculture and Fisheries:**

Seaweeds are used in aquaculture as a food source for various species of fish and shellfish. They contribute to the growth and health of these organisms, supporting sustainable aquaculture practices(Theuerkauf et al., 2022)

- **Bioremediation:**

Some seaweeds can absorb and collect heavy metals and other pollutants from the water. This feature makes them useful in bioremediation efforts to enhance the water quality in contaminated areas (Wastewater et al., 2022)

- **Agar and Carrageenan Production:**

Agar and carrageenan, two types of polysaccharides extracted from seaweeds, are widely used in the food industry. They serve as gelling agents, stabilizers, and thickeners in a variety of food products, including desserts, dairy products, and processed meats(Lomartire & Gonçalves, 2022b)

- **Alginates in Industry:**

Alginates, another type of polysaccharide found in seaweeds, are used in various industrial applications. They are employed as thickeners in the production of paints, cosmetics, and pharmaceuticals. Alginates are also used in wound dressings for their ability to absorb and retain moisture(Sahoo & Biswal, 2021)

- **Biofuel Production:**

Seaweeds are being studied as a potential source of biofuel. Certain seaweeds have high growth rates and carbohydrate content, making them a suitable feedstock for bioenergy production (Maneein et al., 2018)

- **Food and Culinary Use:**

Seaweeds have a long history of culinary use in many Asian countries. They are rich in vitamins, minerals, and other nutrients. Dried seaweeds, such as nori and Kombu, are commonly used in sushi, soups, and various dishes for flavour and nutritional benefits(Rogel-Castillo et al., 2023)

- **Pharmaceutical Applications:**

Seaweeds contain bioactive compounds with potential pharmaceutical applications. These include anti-inflammatory, antiviral, and antioxidant properties. Extracts from certain seaweeds are being explored for their medicinal potential in areas such as wound healing and anti-cancer research(Cho et al., 2022)

1.6 Importance of seaweeds in DM:

The potential importance of seaweeds in helping control diabetes mellitus:

- **Dietary Fiber Content:**

Seaweeds, such as brown algae, are rich in soluble fibers like alginate. Dietary fiber plays a crucial role in managing diabetes by slowing down the absorption of sugars, thus preventing rapid spikes in blood glucose levels after meals(Rasyid, 2017)

- **Low Glycemic Index:**

Foods with a low glycemic index are beneficial for individuals with diabetes as they have a smaller impact on blood sugar levels. Seaweeds, being low in carbohydrates and high in fiber, may contribute to a lower glycemic response when included in meals(L. W. Lu & Chen, 2022)

- **Inhibition of Enzymes:**

Some seaweed chemicals have been demonstrated to block enzymes responsible for carbohydrate digestion and absorption, such as alpha-amylase and alpha-glucosidase. Slowing down these processes, seaweeds may help manage postprandial blood glucose levels (Lomartire & Gonçalves, 2022a)

- **Mineral Content:**

Seaweeds are high in minerals including magnesium and chromium, which aid in insulin action and glucose metabolism. Including seaweeds in the diet may help to maintain healthy levels of these minerals (Singh et al., 2016)

- **Polysaccharides:**

Seaweeds are rich in various polysaccharides, such as Fucoidans and alginate. Fucoidans, in particular, have been studied for their potential anti-diabetic effects. These compounds may exert their influence by improving insulin sensitivity, reducing inflammation, and modulating glucose metabolism (He et al., 2023)

- **Dietary Fiber and Blood Glucose Control**

Certain seaweeds have a high fibre content, including soluble fibres such as alginate, which may help with blood glucose control. Dietary fibre delays the digestion and absorption of carbs, resulting in a more gradual increase in blood sugar levels following meals (L. W. Lu & Chen, 2022)

- **Antioxidant Compounds:**
  Seaweeds include a variety of antioxidants, including vitamins (such as C and E) and polyphenols. Antioxidants aid in combating oxidative stress, which is higher in diabetes and contributes to consequences like cardiovascular disease and neuropathy (Ivanova et al., 2016)
- **Anti-Inflammatory Properties:**

Chronic low-grade inflammation is associated with insulin resistance and the development of type 2 diabetes. Some compounds found in seaweeds, such as phlorotannins, have demonstrated anti-inflammatory effects, potentially mitigating the inflammatory processes involved in diabetes progression (Jaworowska & Murtaza, 2023)

- **Impact on Gut Microbiota:**

Emerging research suggests that the composition of the gut microbiota plays a role in metabolic health, including insulin sensitivity. Certain components in seaweeds, such as prebiotic fibers, may promote the growth of beneficial bacteria in the gut, positively influencing overall metabolic function (Zang et al., 2023)

- **Insulin-Mimetic Effects:**

Some studies have indicated that certain seaweed extracts may possess insulin-mimetic properties, meaning they can act similarly to insulin in promoting glucose uptake by cells.

This could be beneficial in conditions where insulin resistance is a key factor, such as type 2 diabetes (Cherry et al., 2019)

- **Lipid Metabolism:**

Dyslipidemia, marked by irregular lipid levels, is a prevalent occurrence in diabetes and plays a role in cardiovascular complications. Seaweeds have been investigated for their potential to regulate lipid metabolism by reducing triglycerides and cholesterol levels (Trigo et al., 2023)

- **Hormonal Regulation:**

Seaweeds contain bioactive compounds that may influence hormonal pathways related to glucose metabolism,they may affect the secretion and action of insulin or other hormones involved in blood sugar regulation(Rengasamy et al., 2020)

CHAPTER-2

LITERATURE REVIEW

Summary: This chapter is about the literature review on the existing literature done on the topic.

2.1 Data sources and searching strategy

We looked for information in different databases like Medline, Google Scholar, and the Google search engine. We searched from the beginning of these databases until September 2023. To find relevant articles, we used specific keywords related to the topic we were interested in, such as "marine algae," "diabetes," "*Nitophyllum marginale*," and "seaweeds."

2.2 Marine biota in India

India's marine biota is a rich and diverse array of organisms that thrive in its vast coastal and oceanic regions, encompassing approximately 15,042 species, including phytoplankton, marine algae, zooplankton, nekton (such as fish), benthos (organisms living on or in the seabed), and corals. With a coastline stretching 8,118 kilometers and an exclusive economic zone (EEZ) of about 2.02 million square kilometers, India is among the world's most biodiverse marine areas. The Andaman and Nicobar Islands are especially notable, hosting the highest fish diversity in Indian waters, with around 1,431 species. This diversity extends beyond fish to include invertebrates, marine mammals, and various plant species that are crucial for ecosystem stability(Marcovecchio et al., 2013). Despite its wealth of marine life, India's marine ecosystems face significant threats from human activities. Climate change-induced ocean warming and acidification, pollution from industrial runoff and plastic, and overfishing have led to population declines, with 50 marine fish species now threatened, including six critically endangered, seven endangered, and 37 vulnerable species (K. R. Saravanan, 2013). These challenges underscore an urgent need for effective conservation strategies. In response, organizations such as the MBAI, National Institute of Oceanography (NIO), and Central Marine Fisheries Research Institute (CMFRI) are advancing conservation efforts through research on sustainable fishing, habitat restoration, and public awareness. Certain marine organisms act as ecological engineers: corals create reefs that house numerous species, mangroves protect coastlines from erosion and serve as fish nurseries, and seagrasses stabilize sediments while filtering pollutants. These organisms not only boost biodiversity but also strengthen ecosystem resilience. Overall, India's conservation efforts reflect a commitment to sustaining marine biodiversity while addressing ecological and economic challenges. Through ongoing research, conservation strategies, and public engagement, stakeholders aim to preserve these ecosystems for future generations (Venkataraman & Wafar, 2005; (Gaps, 2020); CMFRI), ensuring ecological balance and supporting coastal livelihoods in fisheries and tourism.

2.3 Marine Ecosystem

India has a huge coastline spanning 7,516.6 kilometres, covering both the mainland and its islands, flanked by the Bay of Bengal to the east. The Indian Ocean is to the south, and the Arabian Sea is to the west (Ganesan et al., 2014). This vast coastal region is carefully divided among nine states and four Union Territories. Gujarat, among states, has the longest coastline, whereas the Andaman and Nicobar Islands have the longest coastline among UTs.The mainland coast can be classified as the West Coast and the East Coast, which are distinguished by unique geomorphological features and diverse coastal and marine habitats (CZI et. al.,, 2012). Biodiversity is important on a national and global basis. The Bay of Bengal and Arabian Sea coastline regions, in particular, serve as abundant fishing grounds in South Asia. India proudly holds the top position globally in exporting marine products to various nations. India's coastal regions support a varied range of marine habitats, which span the whole Indian peninsula and include the two principal island clusters. The West Coast is distinguished by its exposed rocky shores, high surf, and headlands, whereas the East Coast has a generally shelving topography with beaches, marshes, lagoons, and deltas. This diversity in coastal landscapes contributes to the richness and uniqueness of India's marine environment. This comprehensive overview showcases the significance of India's diverse coastline, not only as a hub for marine biodiversity but also as a key player in global marine product exports (Prizomwala et al., 2022)

2.4 Marine diversity: Tamil Nadu

Tamil Nadu has a wide range of ecosystems, including marine coastal systems in the Gulf of Mannar and lush terrestrial evergreen forests in the Western Ghats (Retnamma et al., 2021) Located between 76° 14' and 80° 21' East longitudes and 8° 05' and 13° 34' North latitudes, the state is recognised as one of India's super biodiverse zones, according to a report by the Tamil Nadu Biodiversity Board (Menon et al., 2010). The state covers 130,058 square kilometres (50,216 square miles), accounting for around 4% of the country's total landmass (Ramesh et al., 2020), (Kumar, 2012)

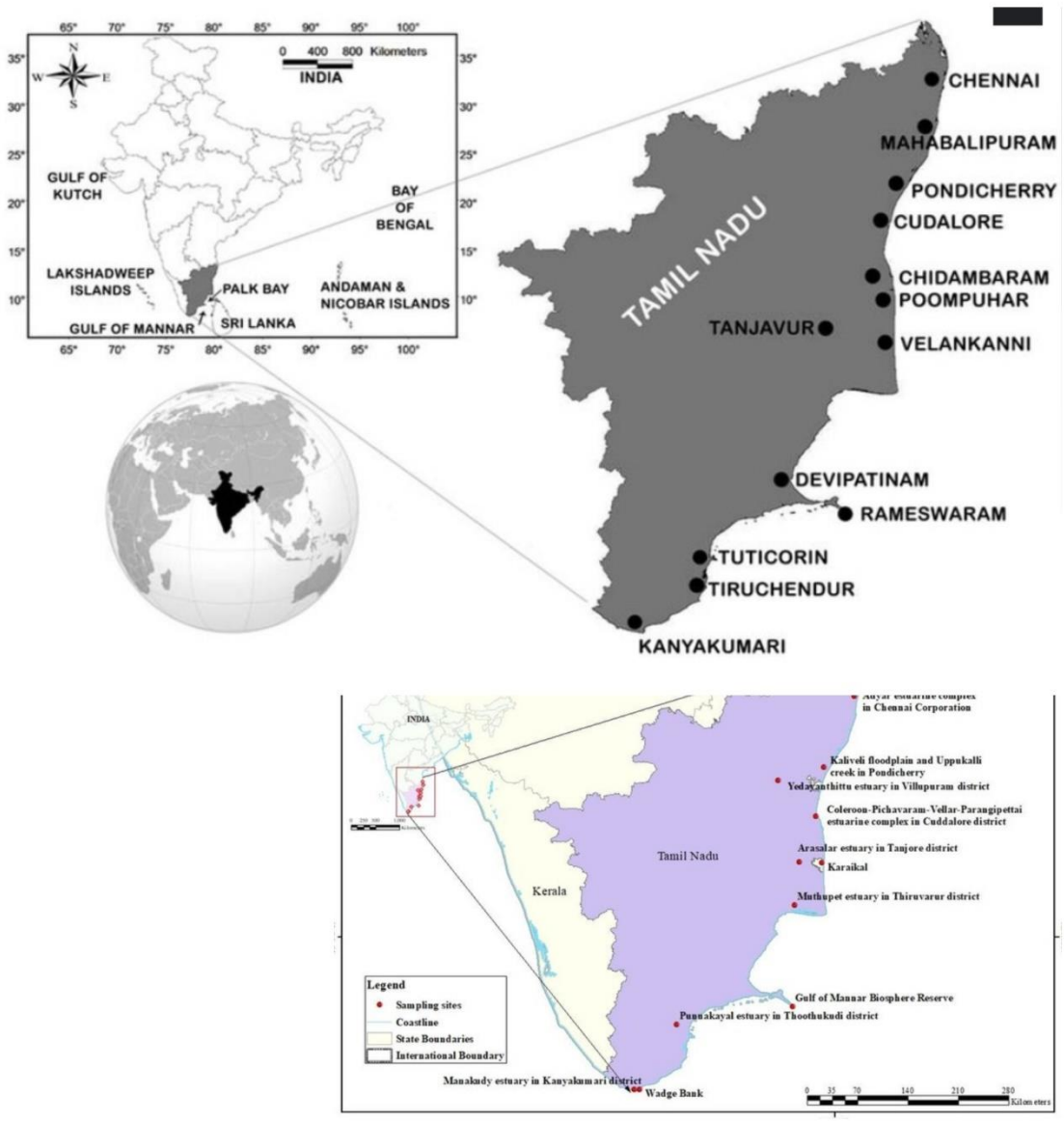

Fig 2: Marine biodiversity of Tamil nadu (Maps of India)

Tamil Nadu is a significant hub of marine biodiversity in India, particularly noted for its rich ecosystems found in areas like the Gulf of Mannar and Palk Bay. This state boasts diverse habitats, including coral reefs, seagrasses, mangroves, and estuaries, which collectively support an impressive array of marine life. Approximately 3,600 species of flora and fauna have been documented in the Gulf of Mannar alone, highlighting its ecological importance. The state's fish diversity is remarkable, with around 1,656 valid species recorded, representing over 51% of India's total fish diversity. However, Tamil Nadu's marine ecosystems face numerous challenges, including habitat destruction from coastal

development, overfishing, and the impacts of climate change. In response to these threats, conservation efforts such as the establishment of the Gulf of Mannar Marine National Park and the Marine Elite Force have been initiated to protect these vital resources. These initiatives are crucial not only for preserving biodiversity but also for sustaining the livelihoods of approximately 1.3 million fishers who depend on these marine ecosystems (Pandey et al., 2023)

2.5 Extraction

Both studies, one by Hussain (2023) and the other by Ali (2021), highlight the crucial role of Soxhlet extraction in various fields. While Hussain emphasizes its efficiency in extracting lipid and fat compounds from solid samples, catering to industries like Agriculture, Pharmaceuticals, Foodstuffs, and Environmental analysis, Ali focuses on its significance in isolating bioactive compounds from seaweeds for promoting sustainable crop production. Despite their different focuses, both studies underscore the versatility and reliability of Soxhlet extraction in extracting valuable compounds for diverse applications (Hussain et.al., 2023), (Ali et.al., 2021)

Both Sutrisna (2020) and Kusumastuti (2019) delve into the application of Soxhlet extraction in the fashion and textile industry, particularly for obtaining natural dyes from various plant sources. Sutrisna's study focuses on the extraction process intended for coloring in fashion industries, while Kusumastuti's research explores advances in Soxhlet extraction methodology, such as temperature and solvent variations, for the production of natural dyes powder specifically tailored for textile applications. Together, these studies highlight the versatility of Soxhlet extraction in the textile sector, emphasizing its role in obtaining natural dyes efficiently for use in fashion and textile products (Sutrisna et al., 2020), (Kusumastuti et al., 2019)

Both Zhang (2018) and Baba (2018) highlight the versatility of Soxhlet extraction in extracting bioactive compounds from natural sources, particularly plants. Zhang's review emphasizes the crucial role of natural products in drug development and discusses various extraction and isolation methods, including Soxhlet extraction, in natural product research. On the other hand, Baba's study specifically demonstrates the effectiveness of Soxhlet extraction in obtaining bioactive compounds from fenugreek seeds and shoots, showcasing its superiority over cold extraction methods. Together, these studies underscore the importance

of Soxhlet extraction in unlocking nature's pharmacy and harnessing bioactive compounds for pharmaceutical and nutraceutical applications (Q. W. Zhang et al., 2018), (Baba et al., 2018)

Gopalasatheeskumar (2018) examines Soxhlet extraction's efficacy in extracting plant metabolites with varying water solubilities while addressing its environmental impact, particularly concerning volatile organic solvent use. This study sheds light on Soxhlet extraction's versatility and potential environmental implications. Khaw (2017) contributes to this discussion by highlighting recent efforts to enhance the efficiency of Soxhlet extraction, emphasizing its role in analytical chemistry for extracting organic compounds from solid or semi-solid samples using volatile organic solvents. Together, these studies provide insights into Soxhlet extraction's effectiveness and its significance in analytical chemistry practices, including considerations for environmental sustainability and solvent usage (Gopalasatheeskumar, 2018), (Khaw et al., 2017)

One of study **Elof (1998)**, discussed Soxhlet extraction role in screening and isolating antimicrobial components from plants is discussed in “Which extractant should be used for screening and isolation of antimicrobial components from plants?’’. highlighting various solvents used based on their polarity (Eloff et. al., 1998)

2.6 Phytochemicals

Recent studies by Kumar (2023) and Chen (2023) highlight the diverse therapeutic potential of phytochemicals and terpenoids, respectively. Kumar emphasizes the health benefits of phytochemical consumption, including diabetes prevention and cardiovascular health, while stressing the importance of optimizing extraction techniques for enhanced yield and bioactivity. On the other hand, Chen explores the anticancer properties of terpenoids sourced from seaweeds, identifying key molecular targets and pathways involved in cancer progression. Their combined findings underscore the significant roles of natural compounds in preventive and therapeutic healthcare, with implications for functional foods, nutraceuticals, and oncological treatments (Kumar et al., 2023), (Chen et al., 2023)

Beltagi (2022) and Heinrich (2021) collectively explore the potential of natural sources for bioactive compounds and their implications for functional food and drug development. Beltagi's review underscores seaweeds' role as sources of bioactive compounds and suggests employing green extraction methods to enhance their utilization. Meanwhile, Heinrich's study highlights the importance of species abundance, particularly in plants with alkaloids, and

emphasizes the need for sustainable sourcing and integrated strategies to ensure long-term viability in natural product development. Together, these works emphasize the significance of leveraging natural sources for therapeutic interventions while addressing challenges such as environmental sustainability and health hazards.(El-Beltagi et al., 2022),(Heinrich et al., 2021)

Leandro (2020) and Arora (2020) collectively underscore the potential of natural sources, particularly seaweeds and phytochemicals, in driving advancements in functional foods and public health. Leandro's review emphasizes the diverse range of phenolic compounds found in seaweeds and their antioxidative properties, suggesting opportunities for commercial applications. Meanwhile, Arora's study highlights the health benefits of phytochemicals in fortifying foods to combat dietary-related diseases, particularly in aging populations. Both studies call for further research and collaboration to optimize extraction methods, ensure safety standards, and maximize the potential of natural compounds for ecological and public health benefits (Leandro et al., 2020), (Arora et. al., 2020)

The review by Rengasamy et al. (2020) underscores the immense potential of seaweeds as a rich source of novel bioactive compounds, particularly in India, highlighting their nutritional, medicinal, and antimicrobial properties. It emphasizes their importance in various fields, including medicine, nutrition, and biotechnology. Cherry et al. (2019) describe the prebiotic potential of complex polysaccharide components in seaweeds, focusing on saccharolytic fermentation. They suggest exploring other seaweed phytochemicals such as polyphenols, carotenoids, and PUFAs for their impact on microbial metabolism and host health. Combining these insights suggests a holistic approach to leveraging seaweeds for pharmaceutical and nutritional applications while advancing understanding of their prebiotic properties(Rengasamy et al., 2020), (Cherry et al., 2019)

Subbulakshmi et al. (2018) emphasized the health benefits of phytochemicals like polyphenols, terpenoids, glucosinolates, and phytosterols found in plants, showcasing their antioxidant, anti-inflammatory, anti-cancer, and anti-microbial properties. Meanwhile, Mohy El-Din and El-Ahwany (2016) shed light on the significance of marine macroalgae as sources of biologically active metabolites for pharmaceuticals and medicine. Their research delved into the biochemical, mineral, and antibacterial properties of seaweed extracts, suggesting their potential for developing novel antibiotics. They also identified active compounds, such as octadecanoic acid and 1,2-benzenedicarboxylic acid, with promising antioxidant properties

for various food and pharmaceutical applications. Integrating these insights highlights the valuable bioactive compounds found in both terrestrial plants and marine sources, showcasing their diverse health-promoting potentials in medicine and health sciences. (Subbulakshmi et al., 2018), (Mohy El-Din & El-Ahwany, 2016)

The study by P & R (2015) highlights the diverse bioactive compounds present in Parthenium hysterophorus leaves, such as alkaloids, flavonoids, terpenoids, and phenolic compounds, supporting its traditional medicinal use for malaria, cancer, and skin burns. Their ethanol extract demonstrated potent antioxidant activity comparable to ascorbic acid and significant antibacterial activity against various microorganisms, indicating its pharmaceutical potential. In contrast, Prajapati et al. (2014) explored the multifaceted properties of carrageenans, particularly their role in gelling, thickening, and stabilizing, which have gained attention in the food and pharmaceutical industries. Toxicological studies have affirmed the non-toxic nature of carrageenan across various seaweed species, fueling ongoing research efforts to capitalize on its versatility for diverse applications. This integration underscores the pharmacological significance of Parthenium hysterophorus leaves and the growing importance of carrageenans as versatile polymers in various industrial sectors(C. G. T. P & R, 2015), (Prajapati et al., 2014)

Liu (2012) explores the historical use of Sargassum spp. in Traditional Chinese Medicine (TCM) for thyroid diseases, highlighting bioactive compounds like meroterpenoids, phlorotanins, and fucoidans with potential immunomodulatory effects relevant to conditions like Hashimoto's thyroiditis. While underscoring its therapeutic potential, further research is needed to elucidate mechanisms and its role in preventing and treating thyroid-related disorders. In contrast, Zhang (2010) discusses the unique polysaccharides found in seaweeds, such as alginate, carrageenan, and fucoidan, known for their antioxidant activity and various functional properties. These complex carbohydrates are commonly extracted for applications in the food and pharmaceutical industries. This combined insight underscores the pharmacological significance of Sargassum spp. in TCM and the industrial applications of seaweed polysaccharides (Liu et al., 2012), (Z. Zhang et al., 2010)

2.7 Antioxidants

Yamamauchi (2024) and Akhter (2024) explored the antioxidant properties of food components and red seaweed, *Gracilariopsis longissima*, respectively. Yamamauchi's study involved extensive DPPH measurements on 169 food components to assess their Trolox

equivalent antioxidant capacities (TEACs) and investigate structural-activity relationships (SARs). Weak correlations between ionization potential (IP) and TEACs suggested predominant single electron transfer-proton transfer (SET-PT) mechanisms. Comparative analyses with H-ORAC assays for approximately 60 compounds revealed distinct TEAC trends for different functional groups, aiding in predictive modeling for molecular design and benchmarks in food science. Meanwhile, Akhter investigated the total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity of *Gracilariopsis longissima* using various solvents. Methanolic and acetonic extracts showed the highest TPC and TFC, with 50% methanolic extracts displaying superior antioxidant activity in both DPPH and ABTS assays. This suggests *Gracilariopsis longissima's* potential as a natural antioxidant source for nutritional and medicinal purposes, emphasizing the importance of solvent selection for optimizing bioactive compound extraction. Further research is needed to identify and characterize specific bioactive compounds in the seaweed extracts (Yamauchi et al., 2024), (Akhter et al., 2024)

Naik (2023) introduced a DPPH-impregnated paper-based disc sensor for detecting vegetable oil adulteration in ghee, offering a simple and convenient method for quality control measures. The sensor, developed through a two-step antioxidant extraction method, effectively identified vegetable oils added at or above 1%, with a sensitivity to detect additions of studied oils at 2.5%. (Naik et al., 2023). In contrast, Cano (2023) highlighted the continued relevance of the ABTS/TAC method despite some drawbacks, emphasizing its speed, minimal processing, versatility across pH ranges, and adaptability to various measurement techniques. The method's ability to assess both hydrophilic and lipophilic antioxidants, along with its compatibility with high-throughput systems, makes it a practical choice for antioxidant assay applications in diverse fields(Cano et al., 2023)

A study comparing phosphine susceptibility in 15 populations of lesser grain borer (Rhyzopertha dominica) across India revealed significant resistance in populations from northern regions compared to northeastern ones. Median lethal concentration values ranged from 0.024 mg/L to 1.991 mg/L, with resistance levels ranging from 1.63 to 82.96-fold compared to susceptible laboratory checks. Furthermore, distinct variations in antioxidant enzyme activities were observed among field populations exposed to phosphine, with peroxidase and superoxide dismutase positively correlated with resistance ratios, while catalase showed a negative association. These findings highlight the differential roles of

antioxidant enzymes in mitigating ox radicals associated with phosphine tolerance in R. dominica (Ranjith et al., 2023)

This study combined research efforts from Portugal and Egypt, highlighting the nutraceutical potential of seaweed extracts and the pharmacological effects of brown macroalgae. Seaweed extracts from Portugal, including *Ulva sp*., *Laminaria ochroleuca*, and *Chondrus crispus*, demonstrated antioxidant properties, with L. ochroleuca extract showing particularly promising results. On the other hand, research on brown macroalgal species from the Egyptian Red Sea coast identified *Polycladia myrica* as a valuable source of various bioactive compounds, such as proteins, lipids, and vitamins, with significant antioxidant activity. These findings underscore the potential of seaweeds as valuable ingredients in functional foods and highlight the importance of further research and commercialization efforts in this field (Amaro et al., 2022), (Ismail et al., 2023)

In 2021, Arun Kumar investigated seaweeds' polysaccharide sulfate content, highlighting *P. pavonica's* superior antioxidant activity and biomedical potential. Meanwhile, Mahendran et al. explored red seaweeds *G. edulis* and *H. valentiae*, emphasizing their pharmaceutical potential in extracted polyphenols. Together, these studies underscore the diverse applications of seaweed-derived compounds in pharmaceuticals and nutraceuticals (Arunkumar et al., 2021), (Mahendran et al., 2021)

In 2020, Chaves explored different methods for quantifying antioxidant activity in 12 plant species, highlighting variations in species categorization and method sensitivity. Meanwhile, Arive et al. (2017) investigated the antioxidant properties of acetone, chloroform, and ethanol extracts from four seaweed species in the Philippines. Their findings suggest significant antioxidant activity in all seaweed samples, with acetone extracts exhibiting the highest activity. Notably, batch 1 of *Caulerpa lentillifera* and *Kappaphycus alvarezii* demonstrated superior antioxidant activity compared to batch 2. These studies underscore the potential of both plant and seaweed extracts as natural antioxidants, emphasizing the importance of method selection and further research in identifying specific antioxidant compounds(Chaves et al., 2020), (Arive et al., 2017)

Ramdani et al. (2017) focused on assessing the antioxidant potential of *Gracilaria bursa-pastoris* extracts from Nador lagoon, Morocco, revealing high phenolic content and significant antioxidant activity in methanolic extracts. Their findings suggest potential applications in medicinal, food, or cosmetic industries, particularly due to the ethanolic

extract's rich phenolic compounds. Conversely, Leelavathi and Prasad (2014) explored various seaweed extracts, including *Sargassum hemiphyllum, Gelidiella acerosa, Gracilaria edulis,* and *Turbinaria conoides*, finding antioxidant activity in methanolic extracts. Moreover, brown seaweeds exhibited higher peroxyl radical scavenging activity compared to red seaweeds, with sulfated polysaccharides from *Fucus vesiculosus* and *Padina gymnospora* showing in vitro antioxidant potential. These studies collectively underscore the diverse antioxidant properties of seaweed extracts, offering potential applications in various industries(Ramdani et al., 2017), (Leelavathi & Prasad, 2014)

Donadee et al. (2011) demonstrate that storage of human red blood cells leads to hemoglobin accumulation, impairing vascular function by scavenging nitric oxide, which induces vasoconstriction upon infusion into rat circulation. This suggests potential mechanisms for endothelial injury and vascular dysfunction. In contrast, Jagetia and Baliga (2004) find that traditional Indian polyherbal drugs like Abana, Chyavanaprasha, and Triphala possess significant nitric oxide (NO) scavenging abilities, superior to Gingko biloba. These findings imply the potential therapeutic efficacy of these polyherbal drugs in combating conditions associated with excessive NO generation, highlighting their diverse pharmacological benefits, including cardio protective and neuroprotective properties(Donadee et al., 2011), (Jagetia & Baliga, 2004)

2.8 Antidiabetic

Seaweeds, rich in secondary metabolites, exhibit potential as antidiabetic agents. Incorporating them into the diet may help manage diabetes by inhibiting glucose-regulating enzymes. Additionally, a study on Nordic seaweed's preventive effects on type 2 diabetes in KK-Ay mice found that *Saccharina latissima* supplementation significantly reduced body weight, HbA1c, and insulin levels while improving HDL cholesterol. This underscores seaweeds' promising role as natural remedies for diabetes management (Vidyashree et al., 2024), (Sørensen et al., 2019)

The studies by Jaber et al. (2023) and Mallhi et al. (2023) provide valuable insights into potential natural remedies for managing diabetes. Jaber et al. demonstrate the potent antidiabetic activity of *Quercus coccifera* methanolic extract, showcasing its efficacy in inhibiting key carbohydrate-metabolizing enzymes and maintaining normal biochemical parameters and body weight. This suggests its potential as a standalone treatment option for diabetes management. Similarly, Mallhi et al. explore the antidiabetic effects of strawberry

extracts in alloxan-induced diabetic rats. They find that treatment with strawberry extract significantly reduces blood glucose levels, serum urea, and creatinine, while increasing body weight, insulin activity, and protein levels, indicating its potential as a natural therapeutic option for managing diabetes-related complications. (Jaber et.al, 2023), (Mallhi et al., 2023)

Unnikrishnan et al. (2022) investigated the anti-diabetic potential of *Ulva reticulata* extracts, revealing significant effects both in vitro and in vivo. The methanolic extract, particularly the chloroform fraction (F4), exhibited substantial inhibition against key carbohydrate-metabolizing enzymes and promoted insulin secretion. Structural elucidation identified five compounds, suggesting potential mechanisms for its antidiabetic activity. Meanwhile, Melakhessou et al. (2021) evaluated the antidiabetic properties of the n-butanol extract from Atractylis fava Desf (BEAF). In vitro assays demonstrated BEAF's inhibitory effects on α-glucosidase and α-amylase activity, while in diabetic rat models, BEAF administration led to a significant decrease in blood glucose levels. These findings collectively suggest the therapeutic potential of both Ulva reticulata and BEAF in managing diabetes and associated conditions (Unnikrishnan et al., 2022), (Melakhessou et al., 2021)

Nie and Cooper (2021) review the potential of polyphenolic compounds in treating type 2 diabetes mellitus (T2DM) by targeting cytotoxic human amylin (hA) aggregates implicated in T2DM pathogenesis. Polyphenols show promise in inhibiting hA aggregation and modulating oxidative stress and inflammation pathways affecting pancreatic islet β-cells. Meanwhile, Odeyemi and Dewar (2020) investigate the antidiabetic potential of polyphenolic compounds from Lauridia tetragona, traditionally used for diabetes treatment in South Africa. Among the fractions obtained, PP4 and PP6 show significant inhibition of α-amylase and α-glucosidase enzymes, with PP4 also enhancing glucose uptake in HepG2 cells. These findings support the potential of L. tetragona as an antidiabetic agent, validating its traditional use(Nie & Cooper, 2021), (Odeyemi & Dewar, 2020)

Naz et al. (2019) investigate the antidiabetic effects of Sedum adenotrichum extract, revealing its ability to normalize blood glucose levels, glycated hemoglobin, and lipid profiles in animal models. The extract, rich in 22 polyphenolic compounds, demonstrates potent α-glucosidase inhibition activity in vitro, suggesting multifaceted antidiabetic mechanisms. Thamada et al. (2019) underscore the importance of exploring secondary metabolites for diabetes treatment in their review. They emphasize the need for alternative strategies and the search for novel bioactive compounds using various in vivo and in vitro

screening models, highlighting experimental induction of diabetes mellitus in animal models and glucose uptake assays for therapeutic research in diabetes management (Naz et al., 2019), (Thamada et al., 2019)

Mohapatra et al. (2018) explore the potential of seaweeds from the east coast of India, including *Gracilariaverrucosa*, *Enteromorphacompressa*, *Ulvafasciata,* and *Turbinariaconoides*, for their antioxidant, hypoglycemic, and antidiabetic properties. Among these, the ethyl acetate extract of *Ulva fasciata* (EAU) exhibits significant antioxidant activity and hypoglycemic effects in normal mice, along with potent alpha-amylase inhibiting properties. These findings suggest *Ulva fasciata* as a potential dietary addition for diabetes management. Gupta et al. (2009) investigate the impact of hot water extract from Helicteres isora fruits on glucose uptake in rodent skeletal muscle cells (L-6 cells). H. isora, known for its traditional use in Indian medicine for its antidiabetic properties, demonstrates significant glucose uptake activity comparable to insulin and metformin, indicating its potential in regulating glucose metabolism for diabetes management (Mohapatra et al., 2018), (Gupta et al., 2009)

The study investigated the synergistic effects of combining glibenclamide, a common anti-diabetic medication, with *G. latifolium* (GL) extract on biochemical parameters in diabetic Wistar rats. Alloxan-induced diabetic rats were treated with either glibenclamide alone, GL extract alone, or a combination of both for 21 days. Results demonstrated that the combination therapy significantly reduced glucose, cholesterol, triglycerides, LDL, and malondialdehyde levels while increasing HDL, glutathione, and catalase activity compared to untreated diabetic rats. This suggests that the combined therapy exhibits potent antihyperglycemic, hypolipidemic, and antioxidant effects superior to individual treatments(ABA, 2016)

Table 1: Review table on seaweeds based on the study

S.No.	Seaweed Type/ Species	Phytochemical Activity		Anti-oxidant Activity				Anti-diabetic Activity (Alloxan/ STZ)	References
		Qualitative (Yes or No)	Quantitative (Yes or No)	DPPH	ABTS	Hydrogen peroxidase Radical Scavenging	Lipid peroxidation		
1.	*Amphiroa fragilissima (red)*	✓	✓	✓	✓	✓	✓	✓	(Easwari & Srisudha, 2018) (Viswanathan et al., 2014)
2	*Centroceras Clavulatum* (red)	✓	✖	✓	✓	✓	✓	✖	(Arunkumar et al., 2021) (Antioxidants, 2017)
3	*Champia parvula*(red)	✓	✓	✓	✓	✖	✖	✓	(*International Journal of Advances in Pharmaceutics Inclusion of Hydrophilic-Lipophilic Balance (HLB) in the Treatment of Psoriasis-A New Approach QR Code*, 2019) (Awah & Verla, 2010) (Subha, 2020)
4	*Gelidiella Acerosa*(red)	✓	✓	✓	✓	✓	✓	✓	(Elsie & Dhanarajan, 2010) (Syad et al., 2016) (Surendran et al., 2019)
5	*Gracilaria arcuat*(red)	✓	✓	✓	✓	✓	✓	✖	(Hidayati et al., 2020)
6	*Gracilaria corticata* (red)	✓	✓	✓	✓	✓	✓	✓	(Rajkumar et al., 2017) (Kannan et al., 2014) (Devi et al., 2022)
7	*Gracilaria edulis(red)*	✓	✓	✓	✓	✓	✓	✓	(G. Silva et al., 2019)
8	*Nitophyllum marginale(red*)	✖	✖	✖	✖	✖	✖	✖	NA
9	*Portieria hornemannii (red)*	✓	✓	✓	✓	✓	✓	✓	(Cojandaraj et al., 2020)
10	*Kappaphycus alvarezii* (red)	✓	✓	✓	✓	✓	✓	✓	(Yulianti et al., 2022)
11	*Turbinaria ornata* (brown)	✓	✓	✓	✓	✓	✓	✓	(Remya et al., 2022) (D. Vijayraja, n.d.)
12	*Turbinaria decurrens* (brown)	✓	✓	✓	✓	✓	✓	✓	(Series, 2019) (Siratantri et al., 2014)
13	*Turbinaria conoides* (brown)	✓	✓	✓	✓	✓	✓	✓	(Grace, 2021) (Santhanam et al., 2019) (Ar et al., 2017)
14	*Stoechospermum marginatum* (brown)	✓	✓	✓	✓	✓	✓	✖	(Deepak et al., 2017)
15	*Spatoglosummargi natum6* (brown)	✓	✓	✓	✓	✓	✖	✖	(Spatoglossum, 2020)
16	*Sargassum wightii* (brown)	✓	✓	✖	✖	✖	✖	✖	(Khan et al., 2022) (Rout et al., 2020)
17	*Sargassum tenerrimum*	✓	✓	✓	✓	✓	✓	✓	(Joy Lindsey et al., 2021) (Agardh,

	(brown)								2022)
18	*Sargassum cistaefolium* (brown)	✓	✗	✗	✗	✗	✗	✗	(D. Silva et al., 2022)
19	*Padina tetrastromatica (brown*)	✓	✓	✓	✓	✓	✓	✓	(Naveen et al., 2021)
20	*Padina pavonica* (brown)	✓	✓	✓	✓	✓	✓	✗	(Čagalj et al., 2021) (Benita et al., 2018)
21	*Padina gymnospora* (brown)	✓	✓	✓	✓	✓	✓	✗	(Rahman et al., 2021) (Praba & Sumaya, 2022)
22	*Padina boergesenii* (brown)	✓	✓	✓	✓	✓	✓	✗	(Kalasariya et al., 2023)
23	*Lobiphora variegata* (brown)	✓	✗	✓	✓	✓	✗	✓	(Thennarasan, n.d.)
24	*Dictyota dichtoma* (brown)	✓	✓	✓	✓	✓	✓	✗	(Rushdi et al., 2022) (El-Katony et al., 2021)
25	*Dictyota bartayresiana* (brown)	✓	✓	✓	✓	✓	✓	✓	(*25, Paper1 Dictyota Bartayresiana*, n.d.) (Rushdi et al., 2022)
26	*Dictyo pterisdelicatula* (brown)	✓	✓	✓	✗	✓	✗	✓	(Magalhaes et al., 2011)
27	*Cystoseira trinoides* (brown)	✓	✓	✓	✓	✓	✓	✓	(Nie & Cooper, 2021) (Sathya et al., 2017)
28	*Valonia utricularis* (green)	✗	✗	✗	✗	✗	✗	✗	NA
29	*Ulva reticulata* (green)	✓	✓	✓	✓	✓	✓	✓	(Unnikrishnan et al., 2022)
30	*Ulva lactuca* (green)	✓	✓	✓	✓	✓	✓	✗	(Zaatout et al., 2019)
31	*Ulva intestinalis* (green)	✓	✓	✓	✗	✗	✗	✗	(Kulkarni et al., 2021)
32	*Halimeda macroloba* (green)	✓	✓	✓	✗	✗	✗	✗	(Basir et al., 2020)
33	*Halimeda gracilis* (green)	✓	✓	✓	✓	✓	✓	✓	(Mohan et al., 2023) (Harborne;, 1987)
34	*Codium tomentosum* (green)	✓	✓	✗	✗	✗	✗	✗	(Marques, 2022) (Rey et al., 2020)
35	*Codium decorticatum* (green)	✓	✓	✓	✓	✓	✓	✓	(MD FAHIM et al., 2022) (Senthilkumar & Jayanthi, 2015) (A. P. P & J, 2022)
36	*Caulerpa taxifolia*	✓	✓	✓	✓	✓	✗	✗	(Bayro et al., 2021) (Box et al.,

	(green)								2008)
37	*Caulerpa sertularioides* (green)	✓	✓	✓	✓	×	×	×	(Fayaz et al., 2021)
38	*Caulerpa scalpelliformis* (green)	✓	✓	×	×	×	×	×	(Review, 2014)
39	*Caulerpa racemosa* (green)	✓	✓	✓	✓	✓	✓	✓	(Mandlik et al., 2022) (Dissanayake et al., 2022)
40	*Caulerpa peltata* (green)	✓	✓	✓	✓	✓	×	×	(Inc ; Jain et al., 1990) (Movahhedin et al., 2014)
41	*Caulerpa laetevirens*(green)	✓	✓	✓	✓	×	×	×	(Antioxidants & Of, 2017)

Research Gap:

Nitophyllum marginale is a red seaweed species that has been notably overlooked in research, despite being part of a recent survey of 41 seaweeds (as discussed above table) species while many seaweeds are extensively studied for their, nutritional, ecological and biotechnological benefits,. No research or documented data has been found on this species, and understanding its ecological role could provide valuable insights into marine biodiversity. The lack of comprehensive studies underscores a significant gap, suggesting that future investigations into its bioactive properties and ecological interactions could offer meaningful contributions to both science and industry.

3.1 AIM

To estimate the antioxidants and antidiabetic effect of alloxan-induced diabetic rats

3.2 OBJECTIVES

- Collection, Identification, and preparation of crude extracts of *Nitophyllum marginale* using different solvents
- Perform preliminary qualitative phytochemical analysis and antioxidant activity of the crude extract
- Identify the antidiabetic effects and its associated parameters of *Nitophyllym marginale* against alloxan-induced pharmacological diabetes in wistar rats.

Chapter-4

MATERIALS AND METHODS

4.1 Sampling location

India's wide coastline displays a wide variety of seaweed vegetation, especially along the coastal waters around Mandapam, Tamil Nadu (DMS Lat: 11º7'37.6428" N, DMS Long: 78º 39' 24.8076" E). A wide range of marine algae, such as brown, red, and green species, can thrive in this location. In this region, seaweeds are important both ecologically and commercially. The most commonly discovered species in the coral reefs of the Gulf of Mannar islands comprised *Dictyota dichotoma, Halimeda gracilis, Padina pavonica, Sargassum polycystum, and Turbinaria ornata* (Gaps, 2020). *Ulva clathara, Ulva fasciata, Caulerpa crasa,* together with *Caulerpa racemosa* while growing more readily in the shallower regions of the coastal zone, which were represented by a cliffy bottom. Such other dominating species are *Sargassum wightii, Caulerpa, Gracilaria, Amphiroa fragilissima, Porteria hornemanii,* and *Nitophyllum marginale* (Veeragurunathan et al., 2022).

4.2 Sample collection and processing:

Nitophyllum marginale was hand-picked from the coastal areas of Gulf of Mannar, Mandapam, Tamil Nadu, India in different three sites (Site1, Site2, Site3) at a distance of 50m each from each site (shown in fig3). The seaweeds are washed with seawater, then with drinking water and finally with purified water. Epiphytes as well as necrotic components were eliminated to ensure that the seaweed remains fresh and its beneficial substances are preserved, The samples were collected and placed in polythene bags, kept under ice at a temperature of 20ºC, and transported to the laboratory (Veeragurunathan et al., 2022). This method prevents disintegration and losing of important compounds, allowing for identification and future reference purposes (Maneein et al., 2018)**.**Seaweed was shade dried for 8-10 days(K. et al., 2008).

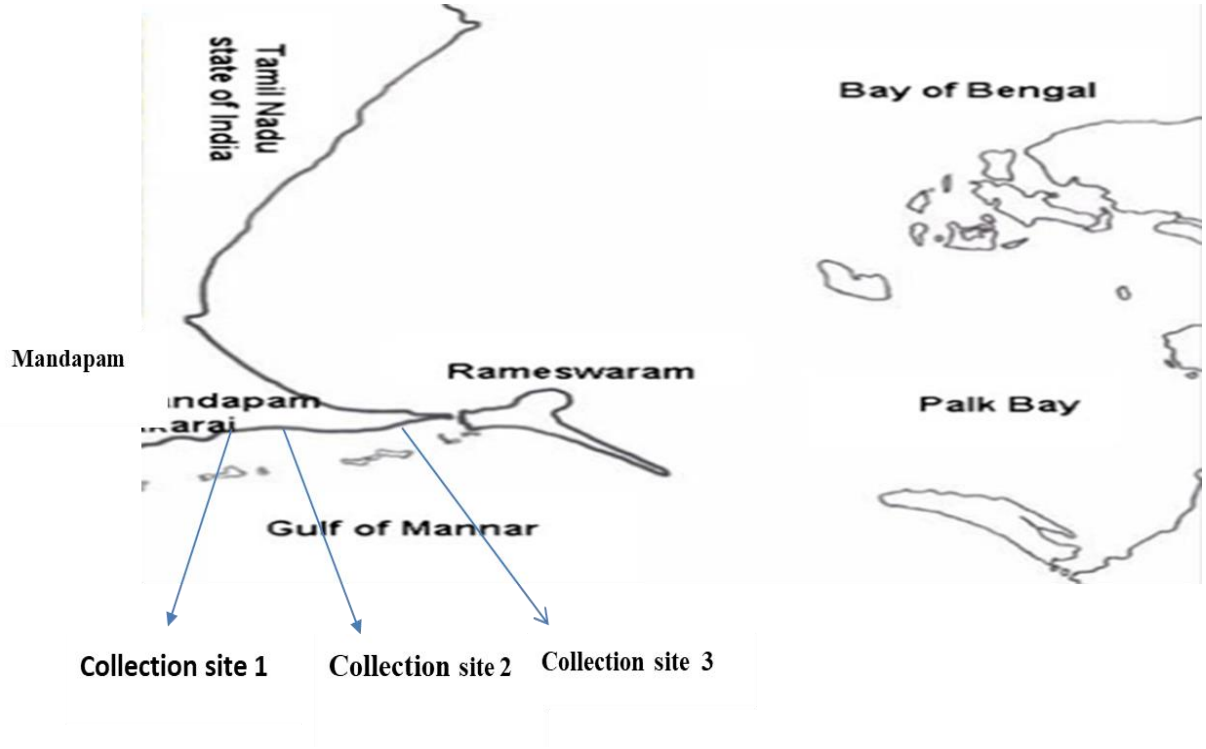

Fig 3: Collection of seaweed from Mandapam (Maps of India)

4.3 Identification:

4.3.1 Taxonomical and Morphological

The live and herbarium sample of Seaweed was sent to Herbasia Biotech., Amritsar, Punjab for identification of Morphological and Taxonomical features.

4.3.2 Molecular Identification (The algal sample was identified using *rbcl* gene sequencing)

a. DNA isolation

The genomic DNA extraction procedure was adjusted with specific modifications to effectively eliminate high polysaccharide content, as outlined by Ramakrishnan et al. (2017). In summary, grinding a substance in the presence of liquid nitrogen with a mortar and pestle smashed down the cell walls. (Alshehri et al., 2019),(Mshiywa et al., 2023). Following this

CTAB method, Lysis buffer (extraction buffer and mercaptoethanol) was added, and the sample was homogenized before undergoing centrifuge at 12,000 revolutions per minute (rpm) for 10 minutes to omit cellular fragments. To top layer, a mixture of absolute alcohol and 3M potassium acetate was added to eliminate polysaccharide contaminants. A solution of chloroform and isoamyl alcohol (24:1 ratio) was introduced in an equal volume, and after vigorous vortexing, the composite were incubated at -20 ºC to 20 mins. Following that, 20 minutes of fractionation at 12,000 rpm were performed. RNase A was then added to the aqueous phase, and the tube was gently mixed by inversion. After 30 minutes of incubation at 37°C, an equivalent volume of chloroform:isoamyl alcohol was added. After vortexing, the tube was incubated at -20 ºC for 20 minutes. After centrifugation, the aqueous phase received additions of 0.8 volumes of isopropanol, 0.1 quantity of 3M sodium acetate, and 0.2% β-mercaptoethanol. After an hour of incubation at -20 ºC, the tube underwent Centrifuge at 12,000 rpm (revolution per minute) for 20 intervals, air-dried and then reconstituted in 50 microliter (µl) of clean water.

b. PCR

To identify in the presence of *rBcl* gene, a Polymerase Chain Reactions (PCR)-based approach was employed. Genomic DNA were initially Extracted from the biological sample utilizing a standard DNA isolation protocol. Specific primers designed for the conserved regions surrounding the *rBcl* gene were then utilized in the PCR reaction setup, along with a high-fidelity DNA polymerase(Mshiywa et al., 2023). The PCR cycling conditions were tailored for *rBcl* gene amplification, involving denaturation, annealing, and extension steps. The resulting PCR products were subsequently analyzed by gel electrophoresis, where the presence and size of the amplified rBcL gene fragment were confirmed under UV light. Optionally, purification of the PCR product and subsequent DNA sequencing were performed for enhanced precision in identifying the targeted gene. This comprehensive PCR-based procedure facilitated the selective amplification and identification of the *rBcl* gene, offering valuable insights into the genetic composition of the examined biological sample.

The PCR was performed using rbcl-F64 and rbcl- R645 primers and amplified product was also analysed using agarose gel electrophoresis

Primer	Sequence	Tm
rbcl-F64	CCTATGCAAAAATGGGATAC	58 ^{o}C
Rbcl-R645	ATCTTTCTTTCCAACGCAT	58 ^{o}C

c. DNA Denaturation

It is used to separate the double stranded DNA in to single strands.

d. Annealing

Depending on the single strand of DNA, primer binding or hybridization occurs in each primer at either the start or finish of the target sequence. Complementary single-stranded DNA can be combined into a single molecule during hybridization.

e. DNA polymerase elongation

The enzyme forms a single-stranded linkage to the primer. DNA uses the existing single strand as a template to duplex and synthesize the complementary strand. DNA strands that have just been synthesized can act as extra templates for complementary strand synthesis.PCR replicates DNA rapidly because it copies both strands, exponentially increasing the number of copies.

f. PCR Master mix preparation

The PCR reaction was carried out in a 20µl reaction mixture with 10µl Takara mix, 1µl of 10µM forward and reverse primers, and 100-200ng of template DNA. The final capacity was formed up of nuclease-free pool. (N. J. Shah, 2019).

g. Electrophoresis and Amplification

To analyze PCR reactions, withdraw 5-10 µl from each reaction and mix with one-sixth capacity of 6X loading buffer. Subsequently, run Specimens on an Agarose gel with gel formation and electrophoresis characteristics adjusted based on PCR product intensity. Post-electrophoresis, stain gels using fluorescent dye SYBR Green I or Ethidium bromide for 20-30 minutes and determine PCR product sizes under UV illumination. Prior to the addition of TaKaRa Taq, briefly mix other kit components, centrifuge, and then add TaKaRa Taq, carefully mixing by Aspirating. Note that the magnesium chloride density in the yielded 10X PCR buffer (Mg2＋) not be optimal, requiring empirical determination by adjusting

concentrations with the provided 10X PCR buffer (Mg2＋ free) and MgCl2 solution(Y.-L. Cheng et al., 2016).

The Amplification reactions took place in a Thermal Cycler (Simpli Amp, Thermo-Fisher) employing conditions that included. The process involved an primal degradation at 95°C for a duration of 5 minutes (N. J. Shah, 2019). Following that, 35 cycles were performed, with denaturation at 95°C for 30 seconds, annealing at 55°C for 45 seconds, and extension at 72°C for 1 minute, respectively. Finally, the Extension stage was completed at 72°C for 10 minutes. After completion, the reaction was halted and kept at 4°C. (Koller, 1957).

The amplified product was sent for Sanger sequencing.

h. Phylogenetic analysis

The rBcl gene sequences of the closest relatives to the RMNM sequence, which were found by NCBI BLAST, were used to construct a phylogenetic tree using MEGA version 11. The greatest likelihood technique was used (Shen et al., 2010). The nodes of the tree were analyzed through bootstrapping. The *rBcl* sequence of *Euptilota formossissima* was used as the outgroup (Kang et al., 2017).

4.4 Dry/ wet yield of *Nitophylllum marginale*

The weight of the *Nitophyllum marginale* was approximated by utilizing a weighing balance. The yield differences between dry and wet samples subjected to incubation before Soxhlet extraction. Performing the calculations to determine the yield for the samples that are wet and dry (H. Zhang et al., 2022)

$$\text{Yield (\%)} = \frac{Weight\ of\ Residues}{Initial\ sample\ weight} \times 100$$

4.5 Extraction

The metabolites from the sample were obtained through Soxhlet extraction, employing diluting agent such as Methanol as well as Chloroform. In the extraction process of seaweed powders, approximately 50 g is carefully weighed and placed into a thimble, which is then positioned within a Soxhlet apparatus. The side arm of the apparatus is insulated with glass wool, and the extraction is carried out utilizing 250 ml of each solvent at a temp. 60°C. As the solvent is flaming using an iso-mantle, it undergoes evaporation, traversing along the

equipment to reach the concentrator (shown in fig 3). The condensed liquid then spills into a repository holding the Thimble, and once the solution level reaches the channel, it flows behind into the vessel. This extraction process is sustained for a total duration of 16 hours, consisting of frequently recurring 10 rotations. Following this, the bio-based dissolving agents are intensified to dehydration under reduced pressure at 30ºC - 35 º C using absorption distillations. The resulting isolates are subsequently desiccated too preserved in freezer for future applications, as outlined by Ganeshan et al. (2007), (Bhuyar et al., 2020). For examination of the phytochemical components, the final residues were retained for qualitative and quantitative analysis of the phytochemical constituents.

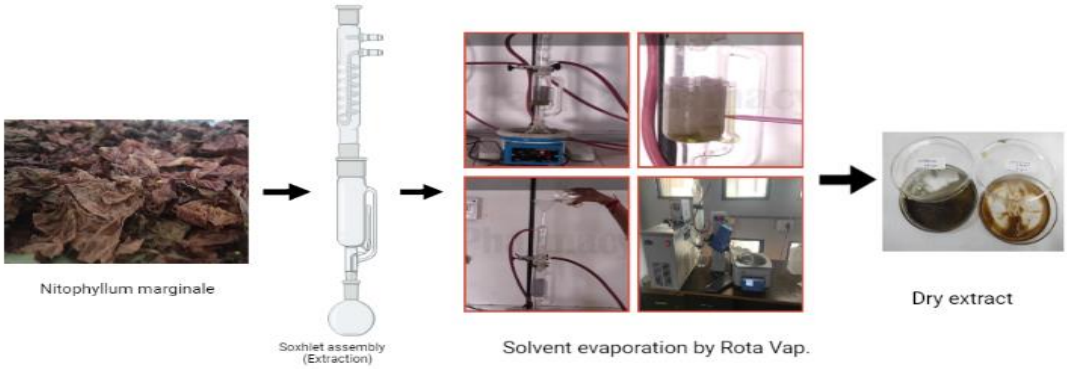

Fig 4: Extraction of Phytochemicals and Solvent Evaporation

4.6 Phytochemicals screening:

4.6.1 Qualitative tests

Phytochemical examination was carried out for the collected seaweed sample. Tests for major phytochemicals such as alkaloids (Mayer's test), flavonoids (ferric chloride study), anthraquinones (Borntrager's test), coumarin (NaOH test), glycosides (Keller-Killani test), phenols (ferric chloride test), saponins (foam test), and carbohydrates (molisch's test) (Sofowora et al., 2013).

a. Detection of Alkaloids

A solution was arrange by mixing 0.355 g of corrosive sublimate and 5 g of iodide of Potassium in water, and when mixed with the seaweed extract and 1% HCl, the resulting filtrate, upon treatment with Mayer's reagent, demonstrated turbidity or precipitation, accompanied by a green color, indicating the presence of alkaloids (Shaikh & Patil, 2020).

b. Determination of Flavonoids

Approximately half of each piece underwent boiling with distilled water and subsequent filtration. Following filtration, a little drips of a 10% ferric chloride resolution was introduced into 2 ml of the resulting filtrate. The formation of a green-blue or violet coloration served as an indicator for the existence of a phenolic -hydroxyl group (Y.-L. Cheng et al., 2016).

c. Detection of Anthraquinones:

1 gram of powdered seaweed was dissolved with 20 mL of chloroform in a dry test tube and boiled in a steam bathe for 5 minutes. During filtration and cooling, the percolate was diluted with an equivalent amount of 10% ammonia solution. The presence of anthraquinones was identified by shaking the mixture while looking for a brilliant pink color in the upper aqueous layer. In a separate test tube, 10 mL of 10% ammonia solution were alloyed with 5 mL of chloroform as a reference. (Deyab et al., 2016).

d. Detection of Coumarin:

The addition of a bit dribbles of alcoholic sodium hydrate to 2 ml of test solution results in the form of a yellow chroma, indicating the presence of coumarin (Dias et al., 2020).

e. Test for Glycoside:

Upon combining 2 ml of the obtain with Glacial acetic acid (GAA) along with one drip each of Ferric chloride ($fecl_3$) and condensed sulfuric acid (H_2SO_4), a reddish-brown pigment appeared at the interface of 2 layers. The detection of a blue-green chroma in the upper layer signified the existence of glycosides (Visweswari et al., 2013).

f. Phenol test

Before 1 ml of Extracts, add 2 mL of deionised water, accompanied by 0.5 mL of washing soda (Na_2co_3) and 0.5 mL of folin Ciocalteau's reagent (FCR). The emergence of a blue/green hue shows the existence of Phenols. (Samar et al., 2022)

g. Saponin test

In a test tube, dissolve 0.5 g of seaweed obtain in 2 ml water heated. Permit it to calm before agitating vigorously to ensure complete mixing. Saponins are detected by the presence of foam (Y. Wang et al., 2022).

h. Steroids test

When 2 ml of the specimen is solvable in 2 ml of chloroform along with 2 ml of intense sulfuric acidic (H_2SO_4) is add-on, a cherry chroma develops in the lower chloroform layer is suggesting appearance of Steroids (R et al., 2017).

i. Tannins

Boil approximately 0.5 g of the algae powder in 20 ml of purified water in a test tube, followed by filtration. Add 1 ml of the extract to the filtrate along with 1 ml of 5% $FeCl_3$. The development of brownish-green coloration show the appearance of tannins (Dey & Ghosh, 2010).

j. Terpenoids testing

Combine 1 mL of the obtain with 2 mL of Chloroform, accompanied by the careful summing up 1.5 mL of conc. sulfuric acid (H_2SO_4), ensuring thorough simultaneous mixing. The emergence of a reddish-brown color at the interfaces signifies the presence of terpenoids (Xu et al., 2023).

k. Test for Carbohydrates

Molisch's test:

The detection of carbohydrates were achieved by dissolving 2ml of extract in a several drips of Alpha-Naphthol, marked by the addition of 2ml of sulfuric acidic adjacent to the test tube. The observation of a purple colored ring indicated the existence of carbohydrates (Dey & Ghosh, 2010).

4.6.2 Quantitative tests

The following biomolecules are identified in the extracts through phytochemical analysis utilizing established quantitative methods.

a. 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 in 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 were then determined in milligrams of gallic acid equivalents (GAE) per gram of dried material (Brighente et al., 2007).

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

b. Flavonoid Analysis

The total flavonoid content was determined using the aluminium chloride colorimetric method described by Lin and Tang (2007). Each sample (0.5 mL) was diluted with 1.5 mL of deionized water. To this mixture, add 0.1 mL of 10% aluminium chloride hex hydrate, 0.1 mL of 1 M potassium acetate, and 2.8 mL of deionized water. After 40 minutes of incubation at room temperature, the absorbance was measured at 415 nm using a spectrophotometer. The standard was quercetin, which was prepared in quantities ranging from 0.05 to 0.45 mg/mL. The total flavonoid content of the plant extracts was reported as milligrammes of Quercetin equivalents (QE) per grammes of dried (Saptarini & Herawati, 2019).

$$Total\ flavonoid\ content \quad = \frac{Concentration\ of\ quercetin \times Volume\ of\ extract}{mass}$$

C. Alkaloids

1 ml of dimethyl sulphoxide (DMSO) was utilized to dissolve 1 ml of seaweed powder, emerged by the additive 1 ml of 2N HCl as well as subsequent filtration. Following filtration, this solving were turnover to a parting funnel, where 5 milliliters of buffer and 5 milliliters of Bromocresol Green (BCG) solution were add-on. The resultant combination were recollected in a 10-milliliter calibrated flask furthermore reduced to amount with chloroform after vigorous shaking with 1, 2, 3, and 4 milliliters of chloroform. Similarly, a series of source caliber solve containing atropine (20, 40, 60, 80, and 100 µg) was ready using the similar method as previously mentioned. The

absorption of both the test and calibration solutions was calculated at 470 nm utilizing a UV/visible spectrophotometer relative to the reagent blank (Fazel et al., 2010).

$$\text{Alkaloids} = \frac{Conc.of\ atropine \times Amount\ of\ alkaloid\ extracted}{Weight\ of\ seaweed} \times 100$$

d. Saponins

The following solutions for the colour development reagents were made: (A) 50 ml of conc. sulphuric acidic as well as 50 ml of ethyl acetate; (B) 0.5 ml of p-anisaldehyde as well as 99.5 ml of ethyl acetate. A 10-milliliter test tube had a two millilitre solution of diluted saponins. The test tube was then closed sac with glass stopper after one millilitre of all the reagent solutions (A) and (B) was add-on. The test tubes was stirred, then permit to develop colour for 10 minutes at 60^0 C in a water bath. It was then permitted to cool for 10 mins. at room temperature in another water bath. At 430 nm, the color-developed solution's absorbance was measured. The absorption were determined utilizing ethyl acetate as a control. Two milliliters of ethyl acetate were added to a test tube as a reagent blank, and it performed similarly to the saponin solution. As standard saponins, diosgenin dissolved in ethyl acetate(Y Uematsu 1, K Hirata, K Saito, 12968 B.C.E.).

$$\text{Saponins} = \frac{Conc.of\ diosgenin \times amont\ of\ saponins\ extracted}{Weight\ of\ seaweed} \times 100$$

e. Terpenoids

Total terpenoids were calculated utilizing the Ghorai et al. (2012) approach. In summary, 200µl of dissolved methanol was introduced to each 2 ml micro-centrifuge tube along with 1.5 ml of chloroform and a known amount of seaweed extract (20 mg). After fully vortexing the sample mixture, let it sit for three minutes. Each 2-ml micro-centrifuge tube should contain 100µl of concentrated sulfuric acid (H2SO4). After that, the assay tubes were incubated for 1.5 to 2 hours in a dark environment at room temperature (30^0 C). Each assay micro-centrifuge tube will have a reddish-brown accelerate in it at the completion of the incubation period. The reaction mixture liquid's supernatant was then cautiously and gently decanted without causing any disruption to the precipitation. After adding 1.5 ml of 95% (v/v) methanol, the composite was eagerly vortexed until the residue was entirely dissolved. The sample was moved from the assay tube to a

colorimetric cuvette, and a blank of 95% (v/v) methanol were utilized to calculate the absorption at 538 nm(Ghorai et al., 2012).

$$\text{Terpenoids} = \frac{Conc.of\ linalool \times amount\ of\ terpenoids\ extracted}{Weight\ of\ seaweed} \times 100$$

f. Total Nitrogen

To determine the Kjeldahl nitrogen content in fertilizers according to AOAC Official Method 978.02, first, accurately weigh 1 to 2 grams of the fertilizer sample into a Kjeldahl digestion tube, then add the appropriate catalyst, typically K2SO4 + CuSO4, followed by 10 mL of concentrated H2SO4. Digest the sample in a Kjeldahl digestion apparatus at temperatures around 350-400°C for 30-60 minutes until the solution becomes clear. After cooling, transfer the contents to a distillation flask with distilled water, add an indicator (phenolphthalein or methyl red), and distill the solution. Titrate the distillate with standardized NaOH solution until the endpoint is reached, indicated by a color change. Record the amount of NaOH used for titration and calculate percentage of nitrogen in the fertilizer sample using the formula:

$$\text{Nitrogen (\%)} = (V \times N \times 14.01 \times 100) / W$$

Where, V is the amount of NaOH used, N is the normality of the NaOH solution, and W is the heap of the fertilizer specimen.

4.7 Antioxidative activity

4.7.1 Scavenging capacity of DPPH radical

The crude powder of algae's free radical seeking activity was determined in vitro using the 1, 1-diphenyl-2-picryl hydrazyl (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).

$$IP = \frac{\text{Abs 515 (control)} - \text{Abs515(test)}}{Abs\ 515\ (control)} \times 100$$

4.7.2 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 kept in the dark for 12-16 hrs, confirming stability over two days at room temperature. Dilute ABTS*+ with absolute ethanol to an 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.

$$\text{IP} = \frac{Abs\ 734\ (control) - Abs734(test)}{Abs\ 734(control)} \times 100$$

4.7.3 Scavenging capability of Nitric oxide

Marcocci et al. employed 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 the reaction, 3 ml of a 10 mM Sodium Nitroprusside solution in Phosphate Buffer Saline were 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 the 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 using concentrations of 0.2, 0.4, 0.6, 0.8, and 1 mg/ml to ensure accuracy. (Abrams et al., 2014).

$$\text{IP} = \frac{Abs\ 546(control) - Abs546(test)}{Abs546(control)} \times 100$$

4.7.4 Lipid peroxidation

The lipid peroxidation caused by the FeSO4-ascorbate system in sheep liver homogenate is measured using thiobarbituric acid reactive substances (TBARS), as described by Ohkawa et

al. (1979). The reaction mixture contains 0.1 ml of sheep liver homogenate (25%) in Tris-HCl buffer (20 mM, pH 7.0; KCl 30 mM; FeSO4 (NH4) SO4.7H2O 0.06 mM), as well as different amounts of synthesised peptides in a final volume of 0.5 mL.After one hour of incubation at 37°C, 0.4 ml is extracted and treated with sodium dodecyl sulphate (8.1%), thiobarbituric acid (0.8%), and trichloroacetic acid (20%). The final reaction mixture is heated, cooled, and centrifuged. The absorbance of the butanol-pyridine layer at 532 nm is measured (Ohkawa et al., 1979). The concentrations of the samples tested are 0.2, 0.4, 0.6, 0.8, and 1 mg/ml.

$$\text{IP} = \frac{Abs\ 532(control) - Abs\ 532(\ test)}{Abs\ 532\ (control)} \times 100$$

4.7.5 Scavenging capability of Hydrogen peroxide

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 H2O2 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 H2O2. The control was the reaction mixture with H2O2 but no extract (Ruch et al., 1989). The sample concentrations tested were 0.2, 0.4, 0.6, 0.8, and 1 mg/ml. The extract's ability to inhibit hydrogen peroxide radicals was determined using the equation below.

$$IP = \frac{Abs\ 232\ (Control) - Abs232(test)}{Abs232(control)} \times 100$$

4.8 Inhibitory activity

4.8.1 Activity of inhibiting yeast α-glucosidase:

The Watanabe et al. (1997) technique was used to examine a test sample's inhibitory activity against yeast α-glucosidase. The catalyst (0.7 U) was diluted in 100 mM phosphate buffer (pH 7.0) with 2 g/L bovine serum albumin and 0.2 g/L NaN3. The substrate solution included 5 mM p-nitrophenyl-α-D-glucopyranoside in the same buffer (pH 7.0). A UV1800 Shimadzu

spectrophotometer (Japan) detected absorbance at 405 nm after combining 1000 µl of enzyme solution and 100 µl of seaweed extract at varying dosages. After 5 minutes of incubation, add 50 µl of substrate solution and incubate for another 5 minutes.The absorbance increased from time zero, and the repressive activity was evaluated as a percentage compared to the blank control (Watanabe et al., 1997). The sample conc. was used 0.2, 0.4, 0.6, 0.8 and 1 mg/ml.

$$IP = \frac{Abs405\ (control) - Abs405\ (test)}{Abs\ 405\ (control)} \times 100$$

4.8.2 Alpha-Amylase activity:

The anti-diabetic effects of seaweed samples were assessed using a modified approach based on the Worthington Enzyme Manual (Worthington, 1993; Kwon et al., 2006). To start, 500µL of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M Nacl) with 0.5 mg/mL of α-amylase enzyme and different amounts (in µg) of seaweed test samples were pre-incubated at 37°C for 10 minutes. After pre-incubation, each tube received 500µL of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) and incubated for 5 minutes at room temperature. The reaction was stopped by the addition of 1.0 mL of dinitrosalicylic acid (DNSA) reagent. The test tubes were placed in a boiling water bath for 5 minutes and then allowed to cool to room temperature (Kwon et al., 2006). The absorbance were compared along controls and blank that composed buffer instead of seaweed sample. The sample concentration to be used 0.2, 0.4, 0.6, 0.8 and 1 mg/ml.

$$\text{Percentage inhibition} = \frac{(Abs.of\ control\ with\ \alpha\ amylase - Abs.of\ test)}{(Abs\ of control\ with\ \alpha\ amylase - Abs\ of\ control\ without\ \alpha - amylase)} \times 100$$

4.9 Cytotoxicity study

4.9.1 MTT assay (3-(4,5-dimethylthiazole-2-yl)-2, 5-diphenyltetrazolium bromide)

3T3 cell line derived from Swiss albino mouse embryo tissue.

On day first, cells are cultured in DMEM media till it got confluent more than 70%. The cells were trypsinized and then it transferred in to 15ml falcon and then centrifuged it for 5 min at 1000 rpm. After removing the supernatent, the cell pellet was placed in fresh medium. Pipette out 10µl from that for the cell counting purpose. According to the counting approx.10,000

cells per well was seeded in triplicates (100µl per well) on 96 well plate and incubated it for 24 hrs. at 37^{o} C and 5% CO_2.

On day second, the media was removed from each well, and the sample was added according to the strength (12.5, 25, 50,100, 200 µg/ml) to each well. Incubated it for 24 hrs at 37^{o}C and 5% CO_2.

On day third, 10µl MTT was added in each well without removing anything from the well and incubated it for 3 hr. After that, each and every well were emptied and 100µl DMSO was added and placed it on the rocker shaker for 20 min. We had taken the absorbance at 540 nm. The percentage of cell viability was estimate by given equation(Dissanayake et al., 2022).

$$\% \text{ Cell viability} = \frac{Mean\ OD\ of\ individual\ test\ group}{Mean\ OD\ of\ control\ group} \times 100$$

4.9.2 Glucose Uptake assay

The L-6 cell line (derived from rat skeletal muscle) is the most well studied cellular model for glucose absorption and GLUT4 translocation. Medicinal herbs can enhance glucose absorption via GLUT4 translocation, as evidenced by in vitro glucose uptake. In vitro models containing skeletal muscle cells and adipocytes are commonly utilised to examine the glucose absorption activity of medicines.

Glucose uptake activity in L6 cells are estimated by the methods described by Guptha et. al. (2010) with slight modifications. Cells are cultured on 12 well plates and incubated for 48 hrs. at 37^{o}C in a CO_2 incubator. When semi- confluent monolayer was formed, the culture was renewed with serum free DMEM containing 0.2%BSA and incubated for 18 hours (serum starved overnight) at 37^{o}C in the CO_2 incubator. After 18 hours, the medium was discarded and cells were rinsed with PBS (pH 7.4) buffer once. They were then treated with 1000µg/ml glucose and test chemical (25, 50, 100 µg/ml) for 1 hour. Glucose uptake was calculated as the difference between the initial and final glucose levels in the incubated medium. The ultimate glucose concentration was determined using a glucose standard graph. The glucose absorption in L6 cells treated with test substances was compared to that in control cells (untreated). If the treated cells demonstrated better glucose absorption compared to control cells, we can presume that the chemical has therapeutic potential.

Prepare the standards by taking different concentrations of glucose (200-1000µg/ml) in each test tube and make up to volume 1 ml with water. Add 4ml of anthrone reagent to each tube and kept in a boiling water bath for 8 minutes. Cool quickly and measure the intensity of the green to dark green colour at 630 nm. Similarly after glucose uptake assay the remaining glucose present in the control and treated wells were assayed by anthrone method as mentioned above (table 11) with the aid of standard curve (Gupta et al., 2009).

4.10 Animal Experimentation

4.10.1 Procurement of animals

The Male wistar (250-300gm) 60 rats were procured from NIPER in Mohali, Punjab, India. The rats were kept in husk –covered propylene cages. Rats were retained in a 12 hours light/dark cycle at 25±2 ^{o}C and 55±10 RH. Rats were inclined a regular pellet feed with free access to water. The design of animal experiment was authorized with protocol no.LPU/IAEC/2023/34 by the Institutional Animal Ethics Committee (IAEC) of the Lovely Institute of technology (LIT Pharmacy), Lovely Professional University (LPU), Punjab, India.

4.10.2 Acute toxicity Studies:

A study on acute oral toxicity was conducted using 24 male Wistar rats following the guidelines outlined by the Organisation for Economic Cooperation and Development (OECD), specifically OECD Guideline 423 for acute oral toxicity testing. Prior to the experiment, the body weights of the animals were individually measured to determine the appropriate dosage. Each rat received a dosage volume of 10 mL per kilogram of body weight. The Wistar rats were provided with standard rat pellets and had access to reverse osmosis (RO) water ad libitum. They were acclimated to laboratory conditions for seven days before the initiation of the testing period. During the testing phase, the rats were housed in a room with a controlled temperature of 24°C and subjected to a 12-hour light/dark cycle.

Twenty-four Wistar rats were randomly divided into four groups of six each. Group II received an initial dose of 50 mg/kg body weight of a diluted extract in normal saline. The rats were observed for general behavioral changes, signs of toxicity, and mortality for the first

4 hours post-treatment, followed by a 48-hour monitoring period. Group III received escalating doses at 48-hour intervals, starting with 300 mg/kg body weight of the extract once there were no signs of toxicity or mortality in group II. Concurrently, group IV received a single dose of 2000 mg/kg of the extract. Additionally, in accordance with OECD guidelines, rats in group I were treated with normal saline to serve as a comparable negative control group (Guideline et al., 2001).

All animals were monitored at least once during the initial 30 minutes of the first 24 hours following vehicle or extract administration, with a specific focus on the first 4 hours post-treatment, followed by daily observations for 14 days thereafter. This monitoring was conducted to detect any clinical or toxicological signs as per OECD recommendations (OECD Guideline for Chemical Testing, Acute Oral Toxicity - Acute Toxic Class Approach, Guideline No. 423, adopted in 2001 by the Organisation for Economic Cooperation and Development in Rome). All observations, including changes in skin and fur, eyes, mucous membranes, and behavioral patterns, were meticulously documented and stored in dedicated files. Incidents such as convulsions, tremors, diarrhea, salivation, lethargy, drowsiness, coma, and mortality were also recorded.

$$\text{Percentage of body weight change} = \frac{\text{Body weight at the end of each week} - \text{initial body weight}}{\text{initial body weight}} \times 100$$

4.10.2.1 Haematology and Serum Biochemistry

At the conclusion of each trial (on the 15th day for acute oral toxicity assessment), the rats were anesthetized using an intraperitoneal injection of 80 mg/kg ketamine (100 mg/ml) and 7 mg/kg xylazine (100 mg/ml). A disposable syringe was utilized to draw a four-milliliter blood sample via cardiac puncture. The collected blood was divided into K2EDTA tubes for hematological analysis (including parameters like hemoglobin, white blood cell count, neutrophils, lymphocytes, monocytes, eosinophils, and basophils) and Plain tubes for biochemical analysis (involving urea, creatinine, albumin, globulin, total bilirubin, alkaline phosphatase (ALP), alanine transaminase (ALT), and aspartate aminotransferase (AST)). To facilitate blood clotting, the blood sample was left in a plain tube at room temperature for 15 to 20 minutes. Subsequently, the tube was centrifuged at 5000 rpm for 20 minutes.

4.10.2.2 Histopathological observations

After blood collection, the rats were euthanized by cervical dislocation, and vital organs (liver, kidneys, and heart) were extracted through a midline incision in the abdomen. The organs were cleaned of excess fat, blotted dry with clean tissue paper, and weighed on a balance. The relative organ weight (ROW) in relation to body weight was calculated and recorded using the following equation:

$$\mathrm{RWO1/4} = \frac{\text{Absolute organ weight}}{\text{Body weight at sacrifice}} \times 100$$

Samples from major organs (liver, kidney, and heart) were examined histologically. They are first fixed in 10% buffered formalin before being routinely treated and embedded in paraffin wax. Glass slides were used to cut paraffin slices (5 μm) and stained with hematoxylin and eosin. The study was completed by an expert pathologist who had no idea which experimental group each part belonged to. The slides were inspected under a light microscope. (Al-Afifi et al., 2018)

4.10.3 Pharmacodynamic study

4.10.3.1 Diabetes induction in Animals

To initiate diabetes, animals underwent an overnight fast followed by an intraperitoneally injection of alloxan monohydrate at a dosage 150 mg/kg. After 3 days post-injection, only rats exhibiting blood glucose levels exceeding 200 mg/dl were selected for further examination(Aba & Edeh, 2019).

4.10.3.2 Blood collection and separation of plasma:

Rat blood was taken by a retro-orbital puncture using a capillary tube and radio immuno assay (RIA) vial. The animal was initially held while having its neck scuffed and its eyes made to bulge. A capillary was inserted dorsally in to the retro- orbital region of the eye, and blood was permitted to enter in to the vial via a capillary tube. The glass tube was kept for centrifuge at 5000 rpm for 15 min with the temperature set to 2-8^{o}C. The clear supernatent (serum/plasma) was removed with a micropipette and stored for processing in a deep freezer at -20^{o}C.

4.10.3.3 Experimental design

The rats will be branched into six groups (n = 6) as follows:

Group I: normal control (non-diabetic), receiving normal saline
Group II: untreated diabetic control, receiving 150 mg/Kg alloxan monohydrate
Group III: diabetic rats treated by the standard diabetic drug glibenclamide (10 mg/kg),
Group IV, V and VI: diabetic rats treated by methanolic extract of *N. marginale* at low, medium and high doses, respectively.
The methanolic extract of *N. marginale* and saline solution will be administered via oral gavage during 21 successive days.
over the course of 21 days, the methanolic extract of N. marginale and a saline solution were provided orally.

4.10.3.4 Measurement of Blood Glucose

The blood glucose concentration of each rat was tested on 1, 7, 14, and 21 days, routinely by drawing blood from the tail vein using the glucose oxidase-peroxidase method (Vijay Kumar & Kiran Dip Gill, n.d.).

4.10.3.5 Body weight analysis

Using a precise balance, the body weight of each rat were measured at 1, 7, 14, and 21 days (Ityodugh et al., 2019).

4.10.3.6 Blood Chemistry Analysis

Following blood Sampling, serum were centrifuged and collected for evaluation of the following parameters: LDL (Friedewald equation), HDL (Watson), triglycerides (Acetyl-acetone), total cholesterol(CHOD-PAP), VLDL (calculated) alkaline phosphates (PNPP AMP),Aspartate transaminase(Reitman & Frenkel), alanine aminotransferase (Reitman& Frankel), total protein (Biuret), and albumin (Bromocresol). Creatinine (Alkaline picrate) and urea (Urease) levels were determined (Mallhi et al., 2023)

4.10.3.7 Collection of organs

Under Wadaan's (2009) instructions, the animals were gently Sacrificed on the twenty-first day via Cervical dislocation. The pancreas, kidney, and liver were surgically removed from the animals for histological examination. The organs were stored in 10% formalin solution for histological analysis at room temperature after being cleaned with normal saline. Using a microscope with magnifications of 10x, 40x, and 100x, Synergy Path Labs Ltd., Jalandhar, India, performed the histological investigation of isolated organs. (Noeman et al., 2011).

4.10.3.8 Antioxidant enzyme assay

a. Catalase (CAT) estimation

In each group, catalase activity was assessed in Liver, Kidney and Pancreas sample. After centrifugation of the homogenized liver, kidney and pancreas, the clear supernatent was collected. One ml of H_2O_2 (30mM) were addition to 1.95 ml of supernatent, and the absorbance was measured for 30 seconds after 15 seconds of internal time (0, 15, 30 sec). The absorbance was calculated with a UV spectrophotometer at lambda max 240 nm(Mazreku et al., 2017)

$$\text{CAT} = \{[2.3 \times log \frac{OD\ initial}{OD\ final}/\Delta t*100]/0.693\}\ /\text{mg of protein}$$

b. Reduced Glutathione (GSH) assay

Butler's methods were used to calculate GSH level; homogenate specimen was mixed with one ml of supernatent tri chloro acetic acid (10% w/v in water) and centrifuged at 1000gfor 10 minutes. 0.5 ml of the supernatent was collected after centrifugation, 2ml of disodium hydrogen phosphate (0.3 M), and 0.25 ml of newly prepared 5, 5- dithio-bis-(2-nitrobenzoic acid (DTNB, 0.001M in 1% w/v sodium citrate) was infused. The reduced glutathione's absorbance was recorded at 412 nm, and the standard plot were employed to determine the strength of GSH (10-100 M)(Tekin & Seven, 2022).

c. Super oxide dismutase (SOD)

It works by reducing the nitro blue tetrazolium (NBT) dye with superoxide radicals produced by the auto-oxidation of hydroxylamine hydrochloride. The reaction mixture includes 1.8 ml sodium carbonate buffer, 750 µl nitro blue tetrazole (NBT-96µm), and 150 µl triton x-100 (0.6%). To commence the reaction, add 150µl of hydroxylamine hydrochloride and incubate for 2 minutes. Then, add 100µl of enzyme extract. The reaction mixture was taken in a test cuvette, and inhibition in the rate of reduction of NBT was measured at 540nm.

.

$$Unit\ activity = \frac{Change\ in\ absorbance/\min(Blank) - change\ in\ absorbance\ /\min(test)}{Change\ in\ absorbance/\min(blank)}$$

4.10.3.9 Oxidative stress markers

a. Determination of hydrogen peroxide (H_2O_2)

The H_2O_2 level will be measured colorimetrically using the 0.1% trichloroacetic acid (TCA) will be used to crush the cells. The homogenate will undergo a 15-minute, 12000-g centrifugation at 4°C. After the layer has been mixed with 10 mM Phosphate buffer (PH 7.0) and 1 mM Potassium Iodide, it will be left in the dark for one hour. At 390 nm, the absorbance of the resultant solution will be determined. A standard curve will be used to compute the concentrations of H_2O_2 (Graf & Penniston, 1980).

b. Determination of malondialdehyde (MDA) content

The TBARS test measures the concentration of Malondialdehyde (MDA) to estimate the degree of Lipid Peroxidation. Supernatent as well as Tris HCl (pH 7.4) were combined in 0.2ml, and the compound was kept at 37^{o}C for two hours. 1ml of 10 % ice-cold TCA was added during incubated, and then the mixture was agitated at 1000g for ten minutes. After 10 minutes in a hot water bath, 1 ml of the supernatent and 1ml of 0.67 % TBA were added, and the samples were allowed to cool before the absorbance at 532 nm was determined. The extinction coefficient of MDA, 0.156M-1, was used in further calculations, and A nanomole of MDA per mg of protein was used to express the final concentration(Zeb & Ullah, 2016).

$$\mathrm{MDA}\left(\frac{\mathrm{mmol}}{\mathrm{mg\ protein}}\right) = (\mathrm{Abs.}\,532 - \mathrm{Abs.}\,600) \times \frac{Volume\ of reaction\ mixture}{Volume\ of\ the\ sample} \times \frac{1000}{£} \times Protein\ conc.$$

Where, £- Coefficient of absorbance (1.53mM)

4.10.3.10 Histopathological analysis

After removal, the remaining tissue was cleaned with regular saline and preserved in 10% formalin. After undergoing additional processing, the preserved specimens were sectioned at a thickness of 4 µm, the specimens were encased in paraffin and underwent staining with hematoxylin and eosin for histological analysis (Kumar et al., 2022).

Haematoxyllin and Eosin stain:

Routine used staining for histopathological sections.Hematoxyllin extracted from heart wood of tree Hematoxylum campachianum. Haematoxyllin is not a nuclear dye so it is oxidized to hematin. In the histopathological staining process, the sections are initially dewaxed using a hot plate and xylene. Hydration follows through graded alcohols (100%, 90%, and 80%), bringing the sections to water. Nuclear staining is achieved with Hematoxylin-Harris for 5-10

minutes. Differentiation is achieved with 1% acid alcohol for 5-10 seconds, followed by a thorough wash in running tap water until the parts become blue. Bluing is achieved by dipping in an alkaline solution, such as ammonia water, following 5 minutes of running water. Sections are stained with 1% Eosin Y for 10 minutes. The process continues with dehydration, clearing, and ultimately, mounting for microscopic examination. This systematic approach ensures the optimal visualization of tissue structures and cellular details in histopathological analysis.

4.11 Statistical analysis.

Data analysis used the Statistical Package for Social Studies version (Graph pad8.0). 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. Additionally, regression and descriptive analyses were conducted. The obtained results were graphically represented for a comprehensive presentation.

Chapter-5
Results and Discussion

5. Results and Discussion

Marine algae contain a diverse range of bioactive nutraceuticals, such as carbohydrates, proteins, minerals, fatty acids, antioxidants, and pigments. The synthesis of these compounds is influenced by various biological and environmental factors, which contribute to the development of both primary and secondary metabolites (Menaa et al., 2021). In India, seaweeds hold significant promise for various applications. The exploitation of phycocolloids like carrageenan, alginate, and agar-agar from seaweeds finds extensive use in industries due to their unique gelling, thickening, and stabilizing properties (Pandey et al., 2023). Additionally, seaweed extracts boast cytotoxic compounds like terpenoids, laminarins, and fucoidans, exhibiting potent anticancer, anti-proliferative, and antimicrobial effects. In the quest to unravel the bioactive potential of marine algae, Research on *Nitophyllum marginale* involves extracting bioactive compounds using methanol and chloroform solvents, with a focus on exploring phytochemicals, antioxidants, and antidiabetic activities in vitro and in vivo. The study aims to uncover the therapeutic potential of *Nitophyllum marginale* for addressing oxidative stress and diabetes-related issues.

5.1 Identification of seaweed

5.1.1 Authentication

The seaweed sources were identified as *Nitophyllum marginale* (kutzing) J.Agardh belonging to class *Delesseriaceae* and genus *Nitophyllum* by phycologist and voucher specimen along with herbarium was submitted with Ref. no. SHB-22/23-01, Amritsar, Punjab.

5.1.2 *rBcl* Gene sequencing and phylogenetic studies

The sequencing of the *rBcl* gene and the phylogenetic study of 18S rRNA were conducted and the results indicated that 213µg/sample have a purity of 1.85 (table 2) further gel image of DNA & ITS amplification showed the presence of DNA on Agarose gel (shown in fig.4)(Chac & Thinh, 2023).

Table2: DNA Quantification

Sample code	Concentration (µg/ml)	Purity (A260/280)
Sample	213	1.85

5.1.3 Gel Image of DNA and ITS Amplification:

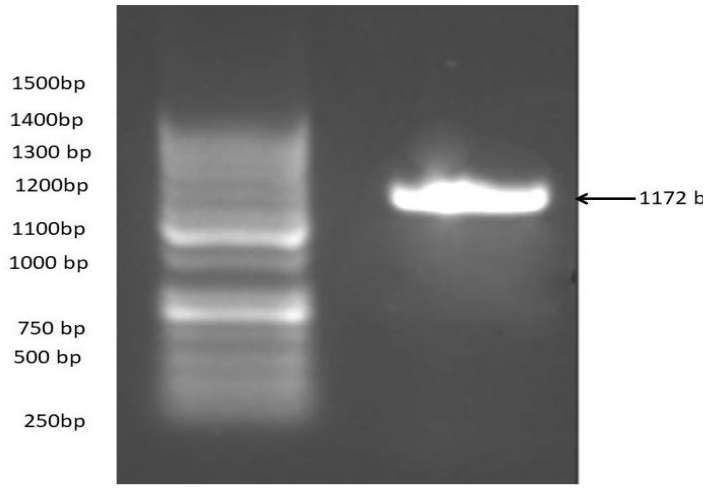

Fig 5a: Agarose gel images of left: total DNA from the sample (with 1Kb DNA ladder Takara) and right: *rBcl* amplified products of the samples (with 50bp DNA ladder Takara)

5.1.4 Sequencing Results for the algae sample (*N.marginale*)

The 1172bp consensus sequence obtained upon initial analysis using NCBI- nucleotide BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi#) were found to belong to ***Nitophyllum marginale*** with 98.32% similarity and 100% query cover (as shown in fig.5b)

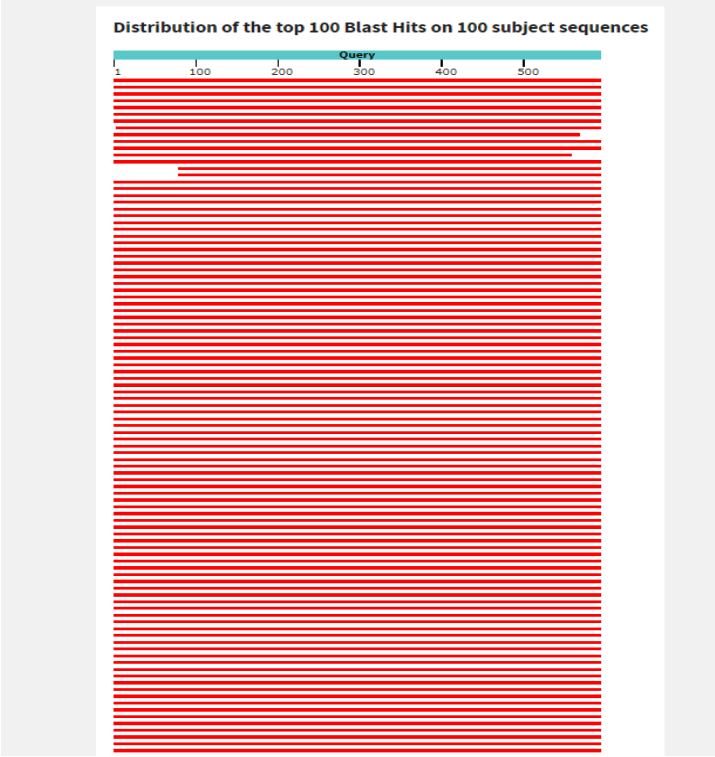

Fig 5b: Similarity percentage of genomic sequences (NCBI Blast:Nucleotide Sequence)

>RMNM rbcl F

CCATATGCAAAAATGGGATATTGGGATCCAAACTlATGTAATTAAGGAAACTGATATTTTAGCATTATTTCGTGT
TACTCCACAACCAGGAGTTGATCCAGTAGAAGCTTCTGCTGCAGTTGCTGGTGAATCATCTACTGCTACTTGGAC
AGTAGTATGGACTGATCTATTAACAGCATGCGATTTATATCGAGCTAAAGCATATAAAGTAGATGCTGTTCCTAA
TACATCTGATCAATACTTTGCTTGCCTCGCTTATGATATCGATTTATTTCTGAAGGTTCTATTGCTAACTTGACA
GTTCTATTATCGGTAATGTATTTGGATTTAAAGCAGTAAAAGCACTAAGATTAGAAGATATGCGTATACCAGTAG
CTTATCTTAAAACATTCCAAGGTCCTGCTACAGGTTTAATTGTAGAACGTGAGCGTATGGATAAATTCGGTCGTC
CATTTTTAGGCGCAACAGTTAAACCTAAACTGGGTTTATCAGGAAAAAACTATGGAAGAGTAGTATATGAAGGTT
TAAAAGGTGGATTAGACTTCTTAAAAGATGATGAAAATATTAATTCTCAACCATTTATGCGTTGGAAAGAAAGAT
TCTTGTATTCAATGGA

>RMNM rbcl R

CCAATTGTACCACCTCCAAATTGAAGTACCAGAACATCATTACCAAGATAATCTAATAACTGGTGCATTTGACCA
CAATGAATACCACCTGAAGCAACTGGTGTTACTTTACGTAAAGATGCCCAATCTTGTTGAAAAAATATACCTTCA
GGTAGATACATAATATCTAGGTATGGTTCTAATAAAGTATCATAGAATCCTTTGATCATTTTTGGATCACCTTCT
AGCTTACCTACTACAGTACCCGCATGAATATGATCAACACCTGCCATACGCATCCATTTACAGATAACTCTAAAG
TTCATTCCATGAATTTTTTGACGAGAGTATTTTGAATTACCAGCACGATGTAAATGTAAAATCATATCATTTTTA
CGAGCCCAAATTCCCATAGTTTGGATAGCAGTATATCCAATTACTAAGTCAATCATGATGATAACTGTGCCTAAT
TGCTTAGCAAATTCAGCTCTTTCATACATATTTTCCATAGTTGCTGCTGTAACATTCATGTAGTGACCTTTTACT
TCACCTGAAAGCAGCAATAGAACGGTTTACTGCTTCCATTGAATACAAGAATCTTTCTTTCCAACGCAT

>RMNM consensus

CCATATGCAAAAATGGGATATTGGGATCCAAACTATGTAATTAAGGAAACTGATATTTTAGCATTATTTCGTGTT
ACTCCACAACCAGGAGTTGATCCAGTAGAAGCTTCTGCTGCAGTTGCTGGTGAATCATCTACTGCTACTTGGACA
GTAGTATGGACTGATCTATTAACAGCATGCGATTTATATCGAGCTAAAGCATATAAAGTAGATGCTGTTCCTAAT
ACATCTGATCAATACTTTGCTTGCCTCGCTTATGATATCGATTTATTTCTGAAGGTTCTATTGCTAACTTGACAG
CTTCTATTATCGGTAATGTATTTGGATTTAAAGCAGTAAAAGCACTAAGATTAGAAGATATGCGTATACCAGTAG
CTTATCTTAAAACATTCCAAGGTCCTGCTACAGGTTTAATTGTAGAACGTGAGCGTATGGATAAATTCGGTCGTC
CATTTTTAGGCGCAACAGTTAAACCTAAACTGGGTTTATCAGGAAAAAACTATGGAAGAGTAGTATATGAAGGTT
TAAAAGGTGGATTAGACTTCTTAAAAGATGATGAAAATATTAATTCTCAACCATTTATGCGTTGGAAAGAAAGAT
TCTTGTATTCAATGGAAGCAGTAAACCGTTCTATTGCTGCTTTCAGGTGAAGTAAAAGGTCACTACATGAATGTT
ACAGCAGCAACTATGGAAAATATGTATGAAAGAGCTGAATTTGCTAAGCAATTAGGCACAGTTATCATCATGATT
GACTTAGTAATTGGATATACTGCTATCCAAACTATGGGAATTTGGGCTCGTAAAAATGATATGATTTTACATTTA
CATCGTGCTGGTAATTCAACGTACTCTCGTCAAAAAATTCATGGAATGAACTTTAGAGTTATCTGTAAATGGATG
CGTATGGCAGGTGTTGATCATATTCATGCGGGTACTGTAGTAGGTAAGCTAGAAGGTGATCCAAAAATGATCAAA
GGATTCTATGATACTTTATTAGAACCATACCTAGATATTAATCTACCTGAAGGTATATTTTTTCAACAAGATTGG
GCATCTTTACGTAAAGTAACACCAGTTGCTTCAGGTGGTATTCATTGTGGTCAAATGCACCAGTTATTAGATTAT
CTTGGTAATGATGTTCTGGTACTTCAATTTGGAGGTGGTACAATTGG

. 5.1.5 Phylogenetic analysis

The rBcl gene sequences of the nearest neighbours of the RMNM sequence were used to generate a phylogenetic tree using NCBI BLAST. The phylogenetic tree was generated in MEGA version 11 using the greatest likelihood method and analysed the nodes using bootstrapping. The *Euptilota formossissima rBcl* sequence was considered as the outgroup (as shown in Fig.6).

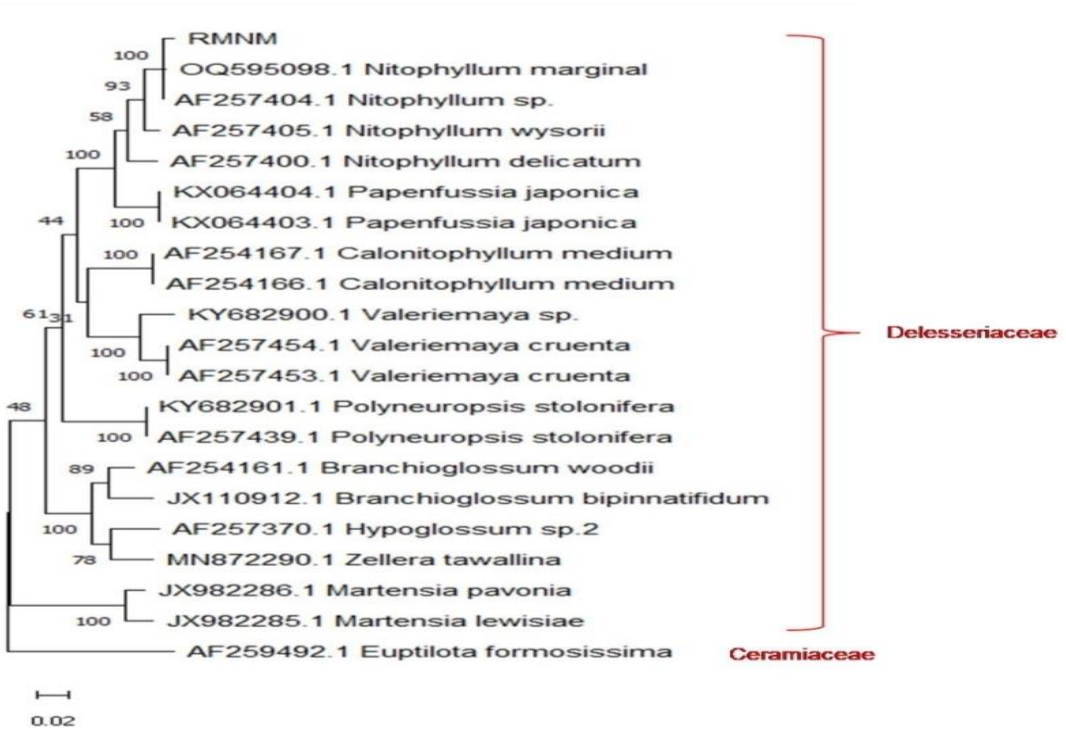

Fig. 6: Maximum-Likelihood phylogenetic tree of RMNM and related sequences

Numerous studies have explored the valuable insights and family tree of different seaweeds, helping us understand where they live and how they're related. Scientists used techniques like DNA sequencing to figure out the family connections, using markers like the *rBcL* gene and 18S rRNA (Mshiywa et al., 2023).

Recent research has deepened our knowledge of seaweed biogeography and phylogenetics, revealing complex spatial distribution patterns and genetic diversity. Despite effective techniques like the 5% Sarkosyl buffer method for DNA extraction, challenges remain in obtaining high-quality DNA suitable for phylogenetic analysis (Mata et.al., 2021). The presence of polymerase inhibitors in seaweed cytosol possess a significant hurdle, hindering PCR amplification (Jin et al., 1997). These inhibitors, linked to seaweed's antiviral and antitumor properties, interfere with enzymatic reactions (Pharmawati et al., 2020). Addressing these challenges requires advanced methodologies and thorough reviews to enhance DNA extraction and amplification. Innovative techniques must be explored to mitigate the effects of polymerase inhibitors while preserving DNA integrity. Collaborative efforts across disciplines are essential to integrate molecular analysis with ecological factors, providing insights into seaweed evolution and ecosystem dynamics. Ongoing research endeavors offer promise for overcoming limitations in seaweed DNA analysis (Pharmawati et al., 2020).

5.2 Total yield of *Nitophyllum marginale*

5.2.1 Dry yield of *Nitophyllum marginale*

The weight of the seaweed was measured by utilizing a weighing balance. Three wet seaweed sample weighing of 900mg, 950 mg and 1000mg were taken and its dry weight was measured, then it was dried at 37ºC for 24hrs. 51 mg, 54 mg and 57 mg of dry yield was obtained. A mean weight obtained from *Nitophyllum marginale* was 54± 3.5 mg while mean percentage of dry yield obtained was 5.7%. (Table 2)

Table 3: Dry yield of *Nitophyllum marginale*

Sample weight wet (mg)	Incubation period	Sample weight dry (mg)	Yield percentage
900	37^0C for 24 hrs	51	5.66
950		54	5.74
1000		57	5.7
	Mean ± S.D : 54 ±3.5		5.7%

5.2.2 Total yield on solvent extraction of *Nitophyllum marginale*

The choice of solvents in the extraction of bioactive compounds is crucial, guided by factors such as low toxicity, ease of evaporation at lower temperatures, favorable physiological absorption, non-disassociation of the extract, prolonged preservation of crude metabolites, and prevention of degradation (Lemes et al., 2022). Seaweed was Soxhlet-extracted by grinding 50g of dried material, packing it into a thimble, and subjecting it to run continuously for 10 hours (8 reflex cycles) of extraction with methanol and chloroform solvents. The resulting extracts were vacuum-dried using a rotary evaporator, air-dried, and stored at 4 ºC for future use (Avinash Kumar Jha, 2022),(Malhotra et al., 2024).About 4.8 and 5.3 g of crude extract were obtained from dried seaweed using chloroform and methanol. A mean ± S.D of 2.7±0.24 (5.4%) and 3±0.25 (6%) (Table 3).

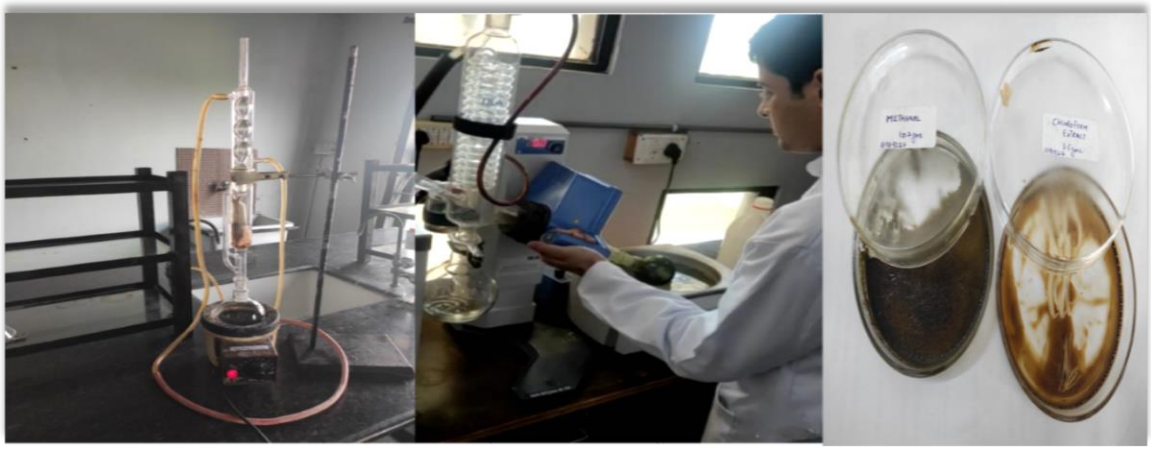

Fig 7: Soxhlet extraction of *Nitophyllum marginale*

The findings of this study underscore the importance of extraction methods and conditions in obtaining optimal yields of bioactive compounds from seaweeds extracts by using Soxhlet extraction methods with varying temperatures, retention time and solvents to evaluate their efficacy in extracting bioactive constituents (Arul kumar et al., 2018). The bioactive compounds identified in seaweeds including those activities, hold significant pharmacological potential (fig 7).

Table 4: Yield of Soxhlet extraction with different solvents

Sample Quantity in gms	Solvents	Mean± S.D	Yield %
50	Chloroform	2.7±0.24	4.8
50	Methanol	3±0.25	5.3

These compounds can potentially be used for the development of novel therapeutics targeting various diseases. This study provides valuable insights in to extraction and identification of bioactive compounds from seaweeds, highlighting the importance of optimizing extraction methods and condition to maximize yield and potency(Silva-Brito et al., 2021). Future research in this area could focus on elucidating the mechanism action of individual bioactive compounds that are useful in the area of interest in the exploring of pharmaceutical potential.

5.3 Preliminary phytochemistry test

Nitophyllum marginale phytochemical characteristics were determined for all the crude extracts obtained from the solvents after condensation in rotary vacuum evaporator(Lakshmanan & Padmanabhan, 2022).

5.3.1 Qualitative examination

The Qualitative analysis of phytochemical constituents in (Table 4 & fig.7), revealing the presence of alkaloids, flavonoids, phenols, terpenoids, saponins, and tannins, indicating the potential bioactivity of the sample(Usman et al., 2009),(Sofowora et al., 2013).

Table 5: Phytochemical analysis of *Nitophyllum marginale*

S.no.	Constituents	Solvents Extraction of *Nitophyllum marginale*	
		Methanol	**Chloroform**
1	Alkaloids	Present	Present
2	Anthraquinones	Absent	Absent
3	Coumarins	Absent	Present
4	Flavonoids	Present	Present
5	Glycosides	Absent	Absent
6	Phenol	Present	Present
7	Saponins	Present	Present
8	Steroids	Absent	Present
9	Tannins	Present	Present
10	Terpenoids	Present	Absent

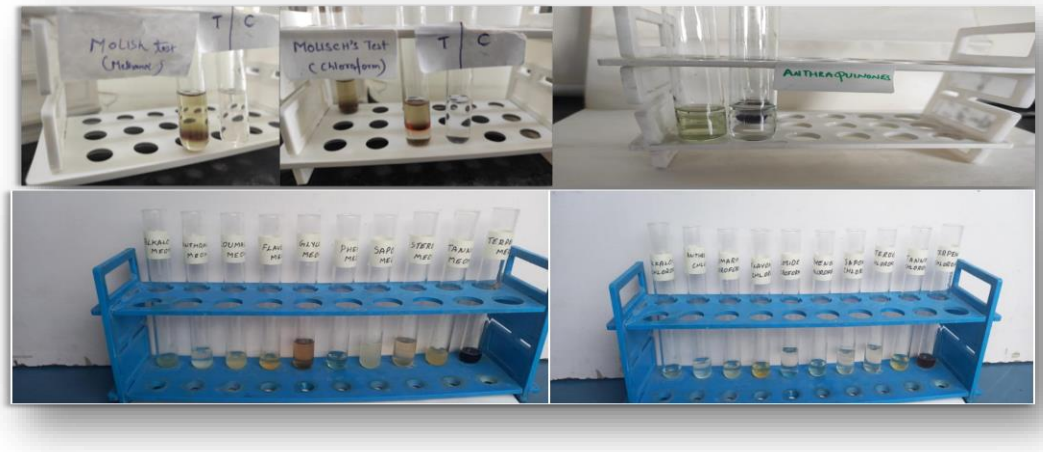

Fig 8:- Phytochemical analysis of Methanolic and chloroformic extract of *Nitophyllum marginale*

5.3.2 Quantitative examination

The extracts were tested for the presence of bioactive compounds by using standard methods in the examination total phenolics, flavonoid, alkaloid, saponins, terpenoids and nitrogen were investigated.

5.3.2.1 Total phenolic content

Seaweeds are a buoyant source of active compounds. It is estimated that there are around 850 known phenolic compounds and most of them are found in marine algae (Leandro et al., 2020). The results of the phenolic content of methanol and chloroform extracts of *Nitophyllum marginale* were 706.5±0.169 and 482.5±0.286 mg GAE/g sample (Table no; 5 Graph 8). The Methanolic extract has high phenolic content followed by chloroform extract. The presence of high phenolic compound could be useful for the prevention of oxidative activities of the extract (Aina et al., 2022) (refer Table 6& fig.9).

Table 6: Extracts of *N. marginale* showing phenolic content

Total Phenolic content	**Methanol** **(** mgGAE/gDE)	**Chloroform** **(** mgGAE/gDE)
Unit	706.5±0.169	482.5±0.286

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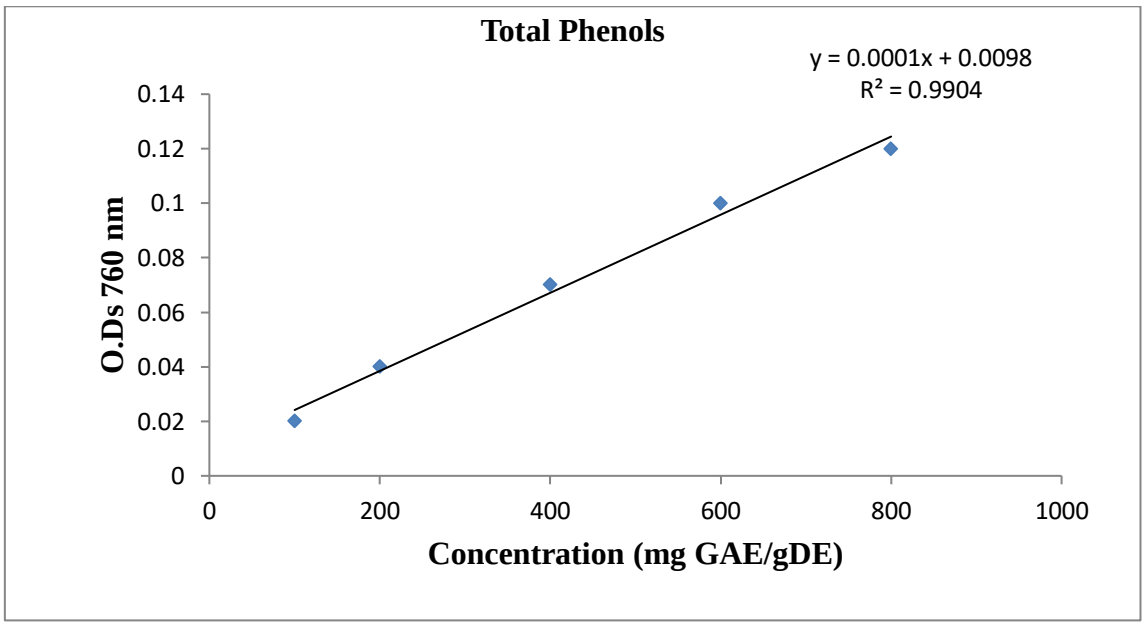


Fig 9: Standard Phenolic Content in mg GAE/gDE.

The phenolic compounds present in seaweed extracts have garnered significant attention due to their potent antioxidative properties and their potential protective effects against cancer and heart diseases(Lakshmanan & Padmanabhan, 2022). In the current investigation, the extract from *N.marginale* exhibited a higher concentration of total phenols compared to other seaweed extracts, indicating its potential as a rich source of these beneficial compounds. Furthermore, the higher phenolic content observed in *N.marginale* suggests its promising role in enhancing antioxidant activity, which is crucial in combating oxidative stress-related diseases. Previous studies have also highlighted the positive correlation between phenolic

content and antioxidant activity, emphasizing the importance of phenolic compounds in scavenging free radicals and mitigating oxidative damage(Hu et al., 2022). Additionally, the antioxidant activity of seaweed extracts, as assessed by various methods such as DPPH and FRAPS, demonstrated a positive relationship with phenolic content. This underscores the significance of phenolic compounds as key contributors to the overall antioxidant capacity of seaweed extract, the high phenolic content observed in *N.marginale* extract holds promise for its potential health benefits, particularly in terms of antioxidative properties(Marinho et al., 2019). Further research into the specific phenolic compounds present and their bioactive effects could provide valuable insights into harnessing the therapeutic potential of seaweed-derived phenolics for combating various diseases associated with oxidative stress. Recent comparison studies have shown that brown seaweeds tends to have higher total phenolic content than red seaweeds.(Abo-Shady et al., 2023) For, Instance, brown seaweeds like *Dictyota spiralis* reported higher in phenolic content while red seaweeds like *pyropia spp. have* lower Total phenolic content values. This difference in phenolic correlates with higher antioxidant activity in brown seaweeds, which makes them more potent for health-related applications like free radical scavenging and oxidative stress prevention(M. M. Ismail et al., 2023). Brown seaweeds (Phaeophyceae) generally exhibited higher total phenolic content than red (Rhodophyceae) and green (chlorophyta) seaweeds. *Fucus vesiculous* and *Ascophyllum nodosum* both brown seaweeds have shown to contain significantly higher levels of phenolic compounds, particularly phlorotannins, which are unique to brown seaweeds. In comparison, red seaweeds *Gracilaria* and *porphyria* species have lower TPC but still contribute to antioxidant activities. However, some species such as pyropia spp., porphyra exhibit moderate total phenolic content indicating variations due to environmental and seasonal influences. These findings suggest that brown seaweeds especially those rich in phlorotannins, offer stronger antioxidant potential, making them more promise for functional food and nutraceutical applications. In contrast, red seaweeds may offer complementary benefits, especially when targeting specific flavonoid-driven health effects (Smita et al., 2022).

5.3.2.2 Total flavonoid content

Present study shows that the amount of flavonoids compounds extracts from methanolic extract of algae was 334±0.48 and from chloroform extract of algae was 374±0.15 mg Quercetin equivalent /DE (refer Table 7 & fig.10)

Table 7: Extracts of *Nitophyllum marginale* represents Total flavonoid content

Total Flavonoid content	**Methanol** mg Quercetin/gDE	**Chloroform** mg Quercetin/gDE
Unit	374±0.15	334±0.48

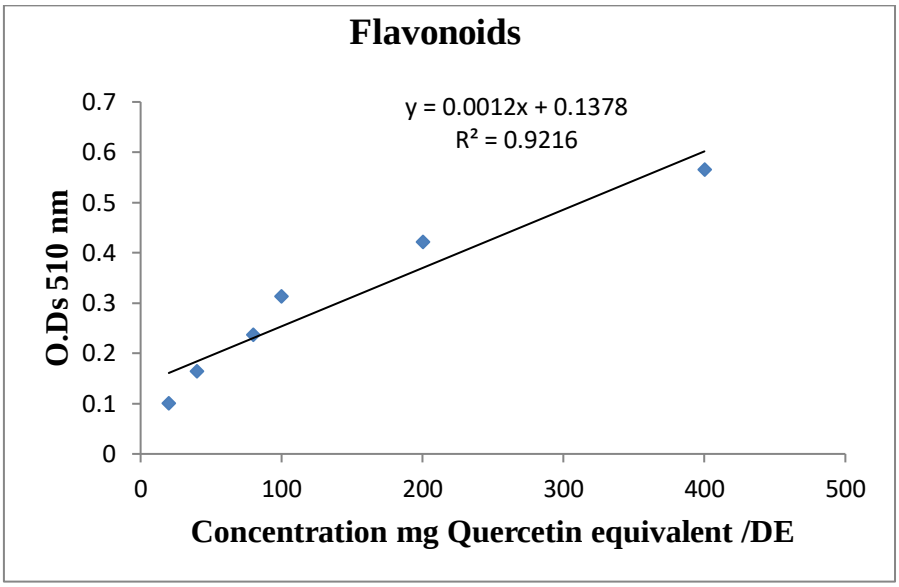


Fig 10: Standard Flavonoid Content in Quercetin equivalent /DE

Flavonoids, a diverse group of phytonutrients found in various plant-based foods, exhibit potent antioxidant, anti-inflammatory, and health-promoting properties. Their chemical structure, consisting of two benzene rings connected by a heterocyclic pyran ring, determines their sub classification and biological activities(Ribeiro et al., 2023). With antioxidant capabilities, flavonoids scavenge free radicals, mitigating oxidative stress and reducing the risk of chronic diseases such as cardiovascular diseases, cancer, and neurodegenerative disorders. Additionally, they exert anti-inflammatory effects by modulating inflammatory pathways, contributing to the prevention and management of inflammatory conditions. Flavonoids' potential in promoting cardiovascular health, preventing cancer, and protecting against neurodegeneration has been extensively studied, highlighting their significance in maintaining overall well-being(Mahmud et al., 2023). Common dietary sources include fruits, vegetables, tea, wine, and cocoa, emphasizing the importance of incorporating flavonoid-rich foods into the diet for optimal health benefits. The comparison of flavonoid content in red, brown and green seaweeds from the Tamil Nadu coast highlight significant variations among species, reflecting their potential health benefits. Brown seaweeds like *sargassum wightii*,

Turbinaria spp. exhibited highest flavonoid content and indicating its strong antioxidant potential and applicable in food and pharmaceutical industries. Red seaweeds like *Gracilaria biknahiae* and *Hpnea valentiae* showcases notable antioxidant properties, making it a valuable source for dietary supplements. Green seaweeds like *ulva lactuca, caulerpa taxifolia* recorded the lowest flavonoid content contributing to its traditional medicinal uses. The data indicate that brown seaweeds generally possess higher flavonoid concentration than their red and green counterparts, aligning with findings from other coastal studies. The trend emphasizes the potential of brown seaweeds like *sargassum wightii* for developing natural antioxidants.(M. M. Ismail et al., 2023) Seasonal variations appear to have a minimal impact on the total flavonoid content across these species, suggesting a consistent source of bioactive compounds throughout the year. The implications for food science and neutaceuticals are significant, as these seaweeds can be integrated in to health products due to their rich antioxidant profiles. All the three groups of seaweeds exhibit beneficial flavonoids, brown seaweeds stand out as particularly promising sources for health applications along the Tamil Nadu coast. Further research is to explore the specific mechanism through which these compounds exert their health benefits(Zhong et al., 2020).

5.3.2.3 Total Alkaloids

The alkaloids content of chloroform and methanol extract of *N.marginale* showed 64.11±0.34 and 196.8±0.82 mg Atropine equivalent/gDE respectively (as shown in Table7 & fig.10).

Table 8: Extracts of *N.marginale* represents Total Alkaloids

Total Alkaloids content	**Methanol** (mg Atropin equivalent/gDE)	**Chloroform** (mg Atropin equivalent/gDE)
Unit	196.8±0.82	64.11±0.34

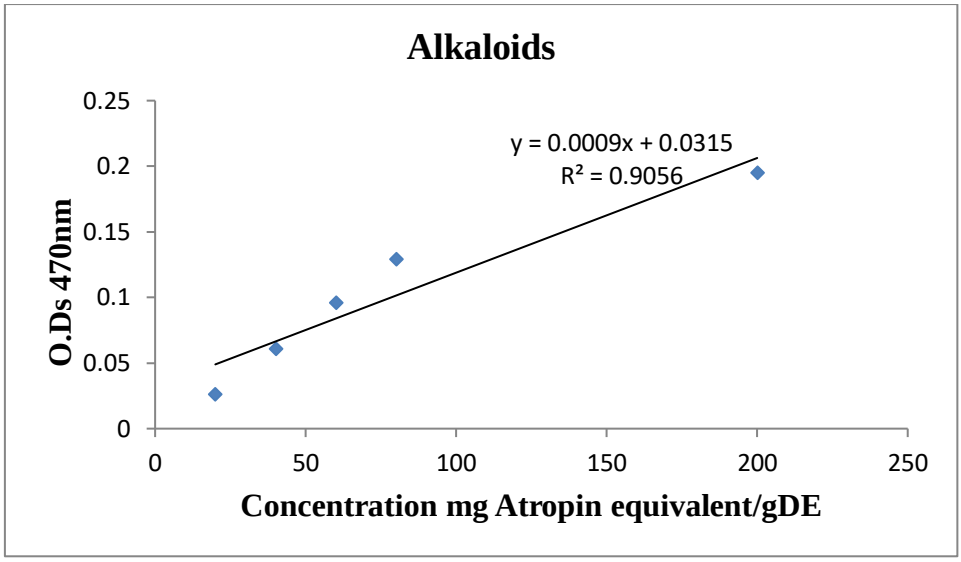


Fig 11: Standard Alkaloid Content in mg Atropine equivalent/gDE

Seaweeds, or marine macroalgae, present a fascinating avenue for research due to their rich composition of bioactive compounds, including alkaloids. These compounds have garnered attention for their potential therapeutic and nutraceutical properties, offering promising avenues for managing various chronic diseases (Meinita et al., 2022).Notably, populations in Asian countries like Japan and Korea, where seaweed consumption is prevalent, exhibit lower incidences of chronic conditions. A recent systematic review delved into the therapeutic potential of seaweed alkaloids, covering a spectrum of diseases including neurodegenerative disorders, obesity, diabetes, cancer, liver disease, cardiovascular ailments, osteoporosis, and arthritis (Ramawat et. al., 2013). The study underscored the importance of further research, both in identifying specific bioactive compounds within seaweeds and conducting clinical studies to elucidate their efficacy in disease management. While seaweed alkaloids hold immense promise, continued investigation is paramount to fully harness their potential and translate findings into tangible clinical applications, ultimately benefiting global health outcomes. The study evaluating Alkaloids in seaweeds from the mandapam region of Tamil Nadu reveal significant differences across various species like Brown seaweeds *sargassum wightii* shown promising alkaloid content, contributing to its recognized antioxidant properties. The methanolic extracts demonstrated higher concentrations compared to other species indicating its potential as a source of bioactive compounds. Turbinaria spp. reported alkaloid level are also notable although specific quantitative data may vary. This species is often highlighted for its medicinal properties. Red seaweeds like *Gracilaria bikhnahiae* and *Hypnea valentiae* contributing to use in traditional medicine. Its extracts have been associated with various health benefits, including anti-inflammatory and anti-oxidant effects, this

species is known for its diverse phytochemical profile, which may include beneficial alkaloids. Green seaweeds *ulva lactuca, caulerpa taxifolia* exhibits lower alkaloid content remains valuable for its nutritional profile and potential health benefits suggesting some bioactive potential but generally less than that of brown and red seaweeds. Overall, brown seaweed appears to possess higher alkaloid concentration compared to red and green counterparts, aliging with findings from other coastal studies. The consistent presence of alkaloids in these seaweeds suggest their role in defense mechanism against herbivores and pathogens. Further research is to explore the specific types of alkaloids present and their mechanism of action, which could enhance their application in nutraceuticals and pharmaceuticals, while all the three groups of seaweeds exhibit valuable alkaloids, brown seaweeds stand out as particularly promising sources for health applications along the mandapam coast. The findings highlight the need for continued exploration of these marine resources to fully understand their potential benefits (Smita et al., 2022)

5.3.2.4 Total Saponins

The saponins content in methanol and chloroform extract of *N.marginale* was shown in Table 8 and fig.11.

Table 9: Extracts of *N.marginale* represents Total saponins

Total Saponins content	**Methanol** (mg Diosgenin equivalent/gDE)	**Chloroform** (mg Diosgenin equivalent/gDE)
Unit	321.8± 1.48	282.6±1.64

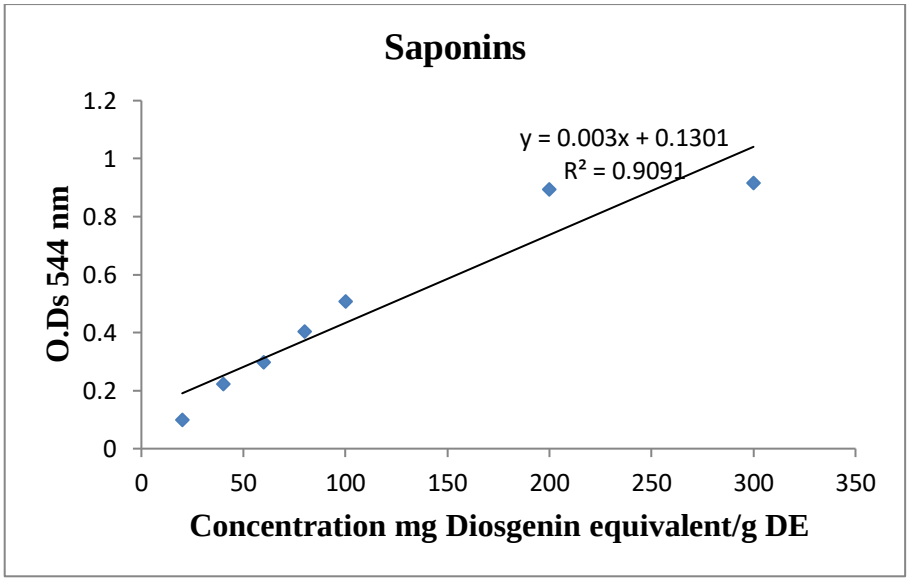


Fig 12: Standard Saponin Content in Diosgenin equivalent/gDE

Saponins, as secondary metabolites found in marine macroalgae, represent a significant area of interest due to their diverse biological activities and potential applications (Feroz et. al., 2018). These compounds, amidst the rich biodiversity of marine ecosystems, have garnered attention for their versatility and promise across various industries. With marine macroalgae divided into distinct classes-Red algae, Green algae, and Brown algae each presenting unique polysaccharides and pigments, the potential for saponin extraction varies across species. Saponins offer a potent source of drugs for pharmaceutical applications, showcasing their relevance in drug discovery and development (El Shafay et al., 2022). However, their extractions from seaweeds possess challenges due to their complex chemical nature, necessitating innovative methodologies. Nonetheless, utilizing dead seaweeds for saponin extraction presents an environmentally sustainable approach, aligning with the ethos of responsible resource utilization. Furthermore, the broad range of biological activities exhibited by saponins underscores their potential in various sectors beyond pharmaceuticals, including food, cosmetics, and agriculture(Vinod kumar et al., 2023). As research into saponins from marine macroalgae progresses, it opens doors to novel applications and underscores the importance of exploring nature's bounty for sustainable solutions to global challenges. The study on saponin content in seaweeds from the mandapam region of Tamil Nadu have highlighted significant variations among different species, emphasizing their potential health benefits. *Sargassum wightii*, a brown seaweed has been reported to contain substantial levels of saponins, contributing to its recognised antioxidant and antimicrobial properties. This species is particularly noted for its efficacy in various bioactive applications

due to its high saponin content. In contrast, red seaweed like *Gracilaria edulis* and *Hypnea valentiae* also exhibit notable saponin levels, which are associated with various health benefits, including anti-inflammatory effects. Green seaweeds such as *ulva lactuca* typically show lower saponin content compared to their brown and red seaweed but still possess nutritional value. Overall, the findings suggest that brown seaweeds, particularly *sargassum wightii* are promising source of saponins for health applications along the mandapam coast, further exploration of their bioactive compounds and potential uses in nutraceuticals and pharmaceuticals(Jayasree et al., 2018).

5.3.2.5 Total Terpenoids

Terpenoids content in methanol and chloroform extract of *N.marginale* was shown in Table 10and fig.13 refers standard linalool equivalent /g DE.

Table 10: Extracts of *N.marginale* represents Total terpenoids

Total Terpenoids content	**Methanol** (mg Linalool equivalent/gDE)	**Chloroform** (mg Linalool equivalent/gDE)
Unit	567.11±0.54	375.81±0.95

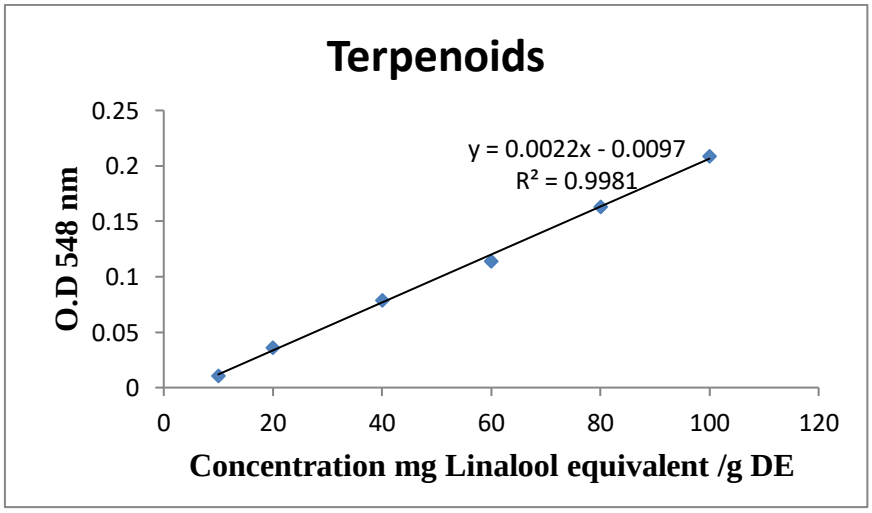


Fig 13: Standard Saponin Content in Linalool equivalent/g DE

The discovery of genes involved in terpenoid biosynthesis within red seaweeds like *Laurencia dendroidea* sheds light on the intricate mechanisms underlying the production of

these complex compounds(De Oliveira et al., 2015). Terpenoids play crucial ecological roles, serving as defense mechanisms against herbivory and biofouling, as well as acting as allele chemicals in competitive interactions within benthic habitats. This new found understanding of the genetic basis of terpenoid biosynthesis opens up exciting avenues for research and application(Abbas et al., 2017). By elucidating the biosynthetic pathways of terpenoids, scientists can explore the potential for harnessing these compounds for various biotechnological purposes(Li et al., 2023). For instance, the heterologous biosynthesis of terpenes from seaweeds could offer sustainable alternatives for producing valuable compounds with ecological or industrial significance. Moreover, the identification of terpene synthase genes in seaweeds provides valuable insights into the diversity of these enzymes and their potential for engineering novel terpenoid products(Cheng et al., 2007), this research not only deepens our understanding of marine ecosystems but also paves the way for innovative approaches in biotechnology and drug discovery, leveraging the untapped potential of terpenoids from seaweeds(Li et al., 2023). Recent investigations in to the terpenoid content of seaweed from various coastal regions, including mandapam, have revealed significant variations among different species highlighting their potential therapeutic applications. Terpenoids known for their diverse biological activities, including anti- microbial and anti-inflammatory properties, are essential phytochemicals found in many marine organisms. Among the brown seaweeds *sargassum species* .and *Turbinaria species* exhibited higher terpenoid concentrations compared to red and green seaweeds. Recent investigations in to the terpenoid content of seaweed from various coastal regions, including mandapam, have revealed significant variations among different species highlighting their potential therapeutic applications. Terpenoids known for their diverse biological activities, including anti-microbial and anti-inflammatory properties, are essential phytochemicals found in many marine organisms. Among the brown seaweeds *sargassum species* and *Turbinaria species* exhibited higher terpenoid concentrations compared to red and green seaweeds. This trend aligns with findings and from other studies that report brown seaweeds often harbouring greater concentrations of bioactive compounds due to their ecological adaptations. Red seaweeds like *Gracilaria species* and *Hypnea valentiae* also show significant terpenoid content but generally at lower levels then their brown counterparts. The observations suggest that while red seaweeds possess valuable bioactive compounds, their terpenoid concentration may not be as pronounced as those found in brown seaweeds. Green seaweeds such as *ulva lactuca* typically exhibit lower terpenoid level. However certain species with in this group can still provide beneficial bioactive compounds that contribute to their nutritional value and

potential health benefits. Overall, the comparative analysis indicates that brown seaweeds are particularly promising source of terpenoids, which may have implications for pharmaceutical applications. The variations observed among different species underscore the need for further research to explore the specific types of terpenoids present and their potential health benefits continued exploration of these marine resources will enhance our understanding of their bioactive compounds and open avenues for developing natural products with therapeutic properties(Choudhary et al., 2023).

5.3.2.6 Total Nitrogen

Total nitrogen content in methanol and chloroform extract shown in table 11 and fig. 14

Table 11: Extracts of *N.marginale* represents Total nitrogen

Total Terpenoids content	Methanol	Chloroform
mg/gDE	123.2±0.57	118.9± 1.27

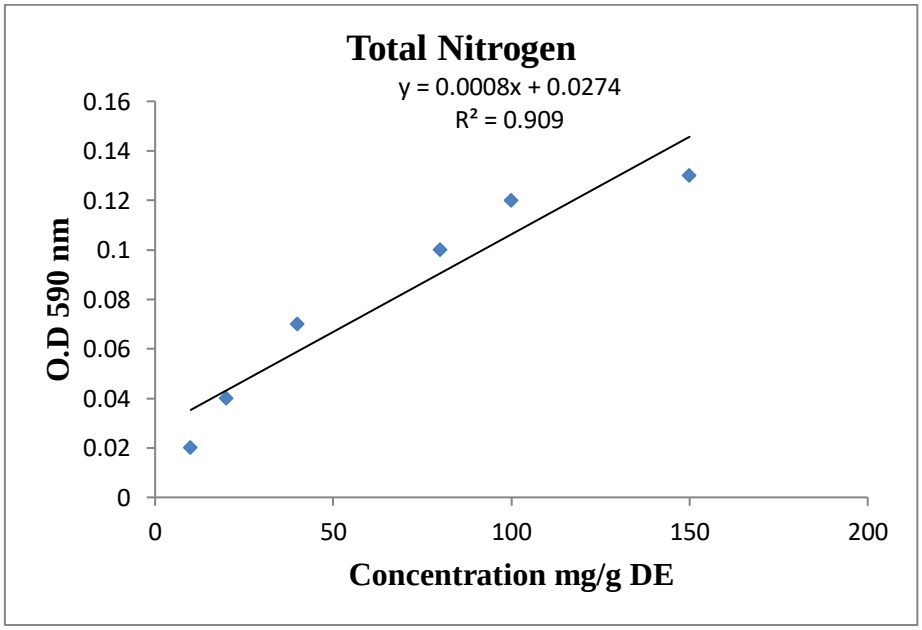


Fig 14: Standard Nitrogen Content in Concentration mg/g DE

The determination of total nitrogen content in seaweeds holds significant importance, especially concerning their potential as a protein source. Historically, the widely adopted nitrogen-to-protein conversion factor of 6.25 has been utilized to estimate protein levels in

seaweeds, assuming that all nitrogen is protein (Angell et al., 2016). However, recent studies have revealed limitations in this approach, leading researchers to seek a more accurate conversion factor specific to seaweeds. Through systematic analysis and meta-analysis of existing literature, it has been established that the traditional conversion factor overestimates protein content. The recommended universal seaweed nitrogen-to-protein (SNP) conversion factor is approximately 5.1, significantly lower than the previously used value(Tibbetts et al., 2016). This adjustment is crucial for obtaining precise assessments of protein content in seaweeds, facilitating their utilization as a sustainable protein source. Furthermore, seaweeds boast a diverse nutrient composition, including protein, lipid, carbohydrate, ash, and energy, highlighting their potential as a valuable nutritional resource (Bito & Watanabe, 2022). Understanding and accurately assessing the nutrient content of seaweeds are pivotal steps towards harnessing their full potential in various applications, ranging from food and feed to pharmaceuticals and biotechnology. The total nitrogen content in seaweeds from the Tamil Nadu coast, particularly around the mandapam region, has been a subject of research due to its ecological and economic significance. Studies indicate that total nitrogen level may vary significantly among different species and are influenced by environmental factors such as nutrient availability and water quality. Brown seaweeds such as *sargassum* and *Turbinaria* generally exhibit higher nitrogen content compared to red and green seaweeds. For instance, research has shown that *sargassum wightii* can have nitrogen levels reflecting its ability to absorb nutrients effectively from the surroundings water. The high nitrogen content is beneficial for its growth and contributes to its role in marine ecosystem by providing habitat and food for various marine organism. In contrast red seaweeds like *Gracilaria edulis* typically show moderate total nitrogen level, often around which supports their use in aquaculture as food source due to their nutrition value. Green seaweeds, such as *Ulva lactuca*, usually have the lowest total nitrogen content. However, these species are still important for their rapid growth rates and ability to provide essential nutrients to coastal ecosystems. The economic implications of these findings are significant, as higher nitrogen content in brown seaweeds can enhance their market value for both food and pharmaceuticals applications. Overall, the variation in total nitrogen content among different seaweed species along the Tamil Nadu coast highlight the importance of these organism in both ecological balance and economic development continued research and sustainable management practices are essential for optimizing the benefits derived from other valuable marine resources(Choudhary et al., 2023).

5.4 Antioxidant activity

5.4.1 Determination using the 1, 1-diphenyl-2-picrilhydrazyl assay.

The ability of the DPPH radical to be eliminated was observed spectrophotometrically at the concentrations mentioned above for the experiment. The methanol extract of *Nitophyllum marginale* resulted in 69.57% inhibition of the DPPH radical, while the chloroform extract yielded 28.92% inhibition (Shown in Table 12& Fig.15).

Table 12: DPPH radical scavenging activity of *N.marginale*

Conc. (mg/ml)	Ascorbic acid	Chloroform extract of *N.marginale*	Methanol extract of *N.marginale*
0	0	0	0
0.2	25.03±0.54	6.39±0.62	10.78±0.38
0.4	39.41±0.52	15.96±0.58	23.86±0.64
0.6	58.3±0.58	22.89±0.66	38.89±0.63
0.8	79.03±0.55	25.15±0.62	51.46±0.66
1	25.03±0.54	28.92±0.77	69.57±0.52

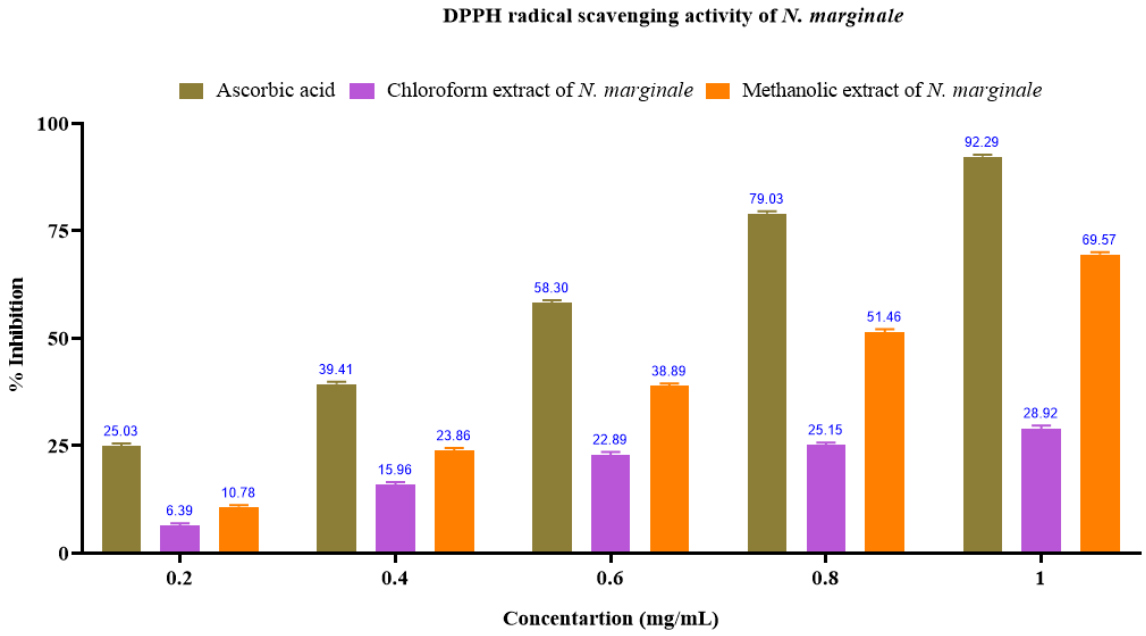


Fig 15: Graphical representation of DPPH radical scavenging activity using methanol and Chloroform extract of Nitophyllum *marginale*

The DPPH (1, 1-diphenyl-2-picrylhydrazyl) assay is a popular method for determining the antioxidant capacity of seaweeds because it analyses compounds' ability to scavenge DPPH radicals, which are extremely reactive molecules containing an unpaired electron. These free radicals cause chain reactions that result in the destruction of cell membranes and cell components such as lipids, proteins, and nucleic acids. In the context of food, free radicals

cause food degradation by lipid oxidation, which impairs the organoleptic characteristics and edibility of food products (V. Sornalakshmi et al., 2021). Seaweeds have been found to exhibit significant antioxidant activity through the DPPH assay. For example, the red seaweed *Gracilaria corticata* has shown a maximum DPPH radical scavenging ability of 74.5% with the ethanol extract, followed by methanol (73.82%), water (73.62%), chloroform (36.9%), and petroleum ether (18.2%). No scavenging activity was noted by the benzene extract. This indicates that different solvents used for extraction can significantly impact the efficacy of antioxidant substances(Devi et al., 2008a). The DPPH assay is also useful for comparing the antioxidant capacity of different seaweed species. For instance, a study found that the red seaweed *Gracilaria corticata* and the brown seaweed *Sargassum wightii* had the highest DPPH radical scavenging activity among six seaweed species tested. The scavenging activity of these seaweeds was significantly higher than that of the standard BHT (butylated hydroxytoluene), indicating their potential as natural sources of antioxidants. The DPPH assay can also be used to evaluate the antioxidant potential of specific compounds in seaweeds. For example, a study found that the phenolic compounds in seaweeds exhibited high DPPH radical scavenging activity, with the methanol extracts yielding the highest phenol content and antioxidant activity(Amaro et al., 2022). This suggests that phenolic compounds in seaweeds may contribute significantly to their overall antioxidant capacity. The DPPH assay is a valuable tool for evaluating the antioxidant capacity of seaweeds, providing insights into their potential as natural sources of antioxidants for various applications, including food preservation and nutraceutical products. The DPPH (1,1-diphenyl-2-picryl hydrazyl) method has been extensively utilized to evaluate the antioxidant properties of various seaweeds collected from Tamil Nadu, particularly from the biodiverse Gulf of Mannar. Among these *Porteria hornemanii* stands out due to its remarkable antioxidant potential. This red seaweed has demonstrated a significant DPPH scavenging activity, with an IC50 value of just 0.19mg/ml, indicating its efficacy in neutralizing free radicals. In laboratory studies it achieved up to 79% inhibition of DPPH, showcasing its ability to protect against oxidative stress, which is linked to various chronic diseases. Another notable species is *Padina gymnospora* a brown seaweed that also exhibits substantial antioxidant activity. In DPPH scavenging capabilities are comparable to those of *Porteria hornemanii*, making it a valuable application in health and nutrition. *Codium tomentosum*, a green seaweed found along the south eastern coast of Tamil Nadu, has shown moderate antioxidant activity in DPPH assays, while its scavenging potential is not as pronounced as that of *Porteria hornemanii*, it still contributes to the overall antioxidant profile of local

marine flora. These findings underscore the rich biodiversity of seaweeds in Tamil Nadu and their potential as natural sources of antioxidants. The bioactive compound presents in these seaweeds could be harnessed for various applications in food and cosmetic industries, where natural antioxidants are increasingly sought after for their health benefits and preservative qualities. As research continues to explore these marine resources the importance of sustainable harvesting practices becomes critical to ensure the conservation of these valuable ecosystems while promoting their utilization in health-related applications(Article & Access, 2022).

5.4.2 Measurement method that uses 2, 2 azino bis (3-ethylbenz thiazoline) -6-sulfonic acid assay

In the experiment, various concentrations of 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS) ranging from 0.2 mg/ml to 1 mg/ml were used. The radical cation ABTS+ was generated using potassium persulfate. The results showed that the highest possible amount of ABTS+, measuring 59.93%, was obtained from the methanolic extract of *Nitophyllum marginale.* On the other hand, the chloroform extract of *Nitophyllum marginale* yielded a slightly lower amount of $ABTS^{+}$, measuring 38.79%. These findings indicate that the methanolic extract had a higher capacity to generate $ABTS^{+}$ compared to the chloroform extract. The different concentrations of ABTS used in the experiment likely influenced the amount of $ABTS^{+}$ generated (shown in Table 13 & Fig.16).

Table 13: ABTS decolouration assay of *N.marginale*

Conc. (mg/ml)	Ascorbic acid	Chloroform extract of *N.marginale*	Methanol extract of *N.marginale*
0	0	0	0
0.2	14.33±0.67	6.42±0.79	9.1±0.67
0.4	33.97±0.52	16.68±0.88	25.25±0.82
0.6	46.79±0.89	23.24±0.82	38.37±0.84
0.8	70.29±0.76	31.65±0.8	46.22±0.78
1	82.05±0.84	38.79±0.74	59.93±0.76

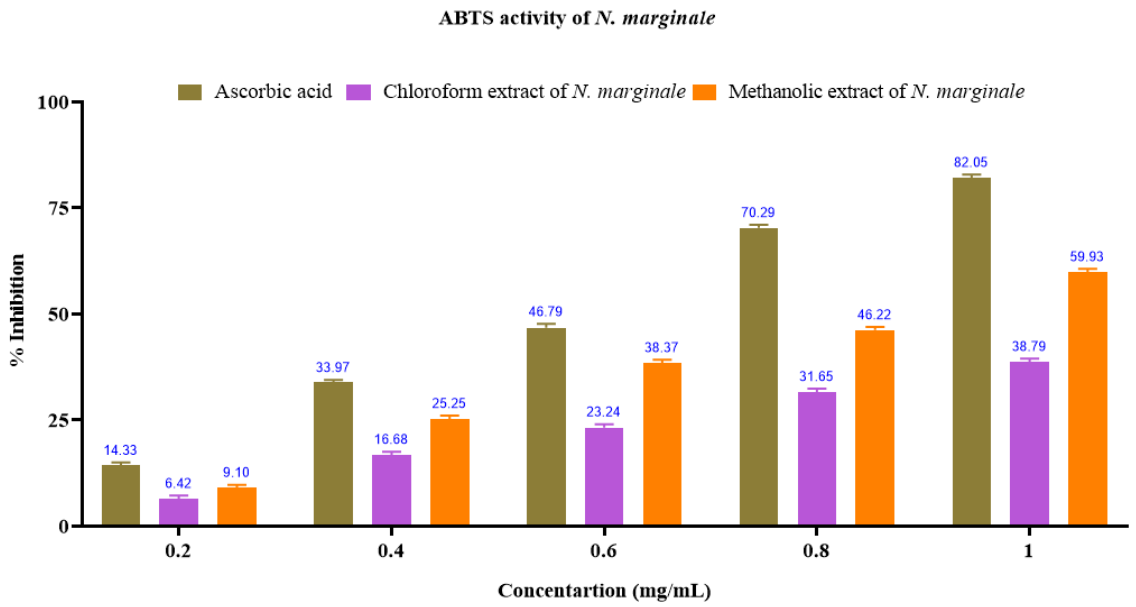


Fig 16: Graphical representation of ABTS radical scavenging using Chloroform and methanol extract of Nitophyllum marginale

The ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) assay is a widely used method for assessing seaweed antioxidant ability. This assay assesses substances' ability to scavenge ABTS radicals, which are extremely reactive molecules with an unpaired electron. The ABTS test is a significant instrument for measuring the antioxidant content of seaweed extracts because it provides insights into their potential as natural sources of antioxidants for diverse uses, such as food preservation and nutraceutical products.

A study on the antioxidant capacities of seaweeds revealed that the ABTS radical scavenging activity was considerably improved at 50% concentrations of methanolic, ethanolic, acetonic, and watery seaweed extracts (Akhter et al., 2024). Specifically, 5 mg/ml of seaweed extract exhibited the finest ABTS-scavenging capacity, with the methanol extract yielding the highest scavenging activity (70.51%, IC50 = 0.032 mg/ml). The acetonic extract also showed significant scavenging activity (61.88%, IC50 = 0.042 mg/ml), while the water extract exhibited moderate activity (47.11%, IC50 = 4.221 mg/ml). The study demonstrates that the extraction solvent and seaweed variety have a substantial impact on the availability of bioactive compounds, influencing the antioxidant activity of the extracts. Another study on the antioxidant activities and phenolic content of three red seaweeds found that the ABTS assay is a promising approach for assessing the free radical scavenging activity of seaweed extracts (Chakraborty et al., 2015).The study reported that the ABTS assay showed a strong correlation with the DPPH assay, which is another widely used method for evaluating the

antioxidant capacity of seaweed, the ABTS assay is a valuable tool for evaluating the antioxidant capacity of seaweeds, providing insights into their potential as natural sources of antioxidants for various applications, including food preservation and nutraceutical products. The extraction solvent and type of seaweed used have a significant impact on the availability of bioactive chemicals, which influences the antioxidant activity of the extracts. The ABTS assay is a valid method for measuring the free radical scavenging ability of seaweed extracts, and it displays a significant association with the DPPH assay. The studies have highlighted the antioxidant potential of various seaweeds through the ABTS assay. For instance, *Porteria hornemani*, a red algae from Tamil Nadu exhibited significant ABTS radical scavenging activity, outperforming other solvent extracts. Methanolic extract showed the highest activity emphasizing the importance of solvent choice in extracting bioactive compounds. *Padina minor* another brown algae, demonstrated 98% inhibition of the ABTS radical, showcasing its potent antioxidant properties. Similarly, research on *Hypnea musciformis* indicated that its ethyl acetate and dichloromethane fractions exhibited high scavenging activities attributed to the presence of phenolic compounds and other bioactive metabolites. Moreover, studies have shown that the antioxidant activity correlates positively with total phenolic content in seaweeds, *kappaphycus alvarezii* and *Gracilaria species* also reinforcing their potential as natural antioxidants(Mei Ling et al., 2013).

5.4.3 Assay for scavenging of nitric oxide

The activity of scavenging nitric oxide was evaluated using methanol and chloroform extracts of *Nitophyllum marginale*. The results showed that the methanol extract exhibited an activity of 52.58 % in scavenging nitric oxide, while the chloroform extract showed a slightly lower activity of 29.12%. These findings indicate that both extracts of *Nitophyllum marginale* possess a moderate ability to scavenge nitric oxide, with the methanol extract displaying a slightly higher activity compared to the chloroform extract across the range of concentrations tested (shown in Table14 & Fig. 17).

Table 14: Nitric oxide scavenging activity of *N.marginale*

Conc. (mg/ml)	Ascorbic acid	Chloroform extract of *N.marginale*	Methanol extract of *N.marginale*
0	0	0	0
0.2	19.17±0.379	9.65±0.407	17.61±0.532
0.4	38.2±0.329	14.16±0.486	28.42±0.639
0.6	65.75±0.339	20.25±0.417	39.85±0.632
0.8	79.91±0.29	23.97±0.486	46.17±0.684
1	90.86±0.42	29.12±0.536	52.58±0.367

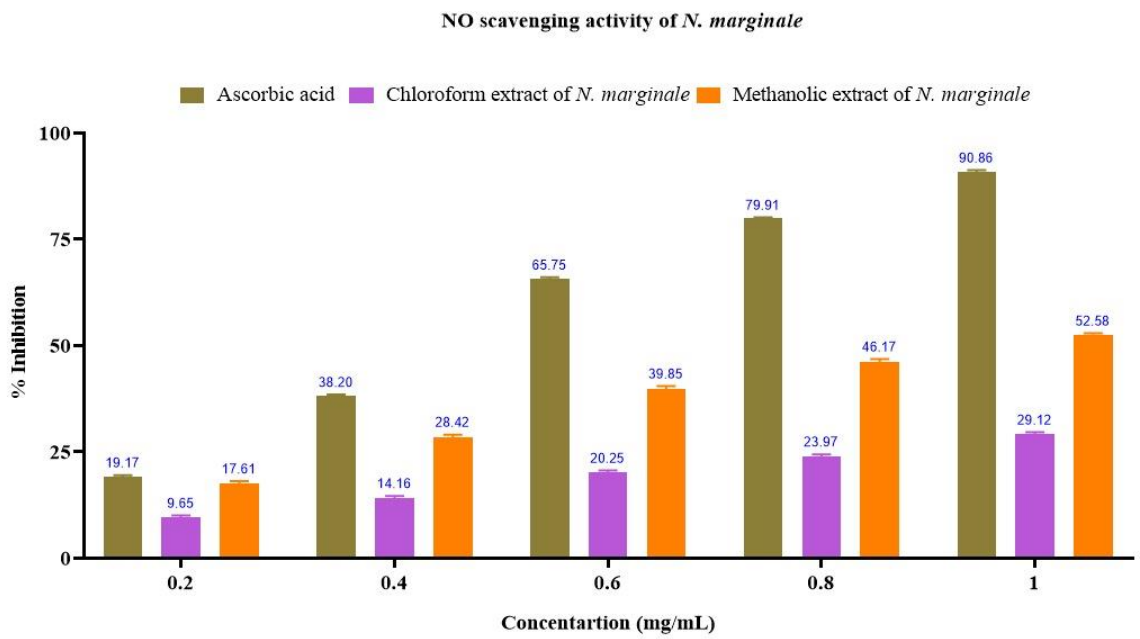


Fig 17: Graphical representation of Nitric oxide radical scavenging activity using Chloroform and methanol extract of *Nitophyllum marginale*

Nitric oxide plays a crucial role in the biological activities of seaweeds, particularly in their antioxidant and immunomodulatory effects. Several studies have highlighted the significance of nitric oxide in the context of seaweed bioactivity: A study demonstrated that fucoidan from *Sargassum autumnale* inhibits potential inflammatory responses via NF-κB and MAPK pathway suppression in lipopolysaccharide. This inhibition includes the down regulation of inducible nitric oxide synthase (iNOS) expression, which is essential in modulating inflammatory responses(Liyanage et al., 2023). Research has shown that seaweeds like *Gracilaria acerosa* exhibit significant antioxidant activity, with *G. acerosa* demonstrating the highest DPPH radical scavenging activity among the seaweeds tested. This scavenging activity is attributed to the suppression of nitric oxide (NO) release, indicating the potential of seaweeds as natural sources of antioxidants with nitric oxide scavenging properties(Devi et al., 2008a).This study explores the immunomodulatory effects of *Caulerpa lentillifera* extracts on nitric oxide production. Nitric oxide (NO) is a key player in immune responses, and the study investigates how seaweed extracts influence NO production and phagocytosis activity in murine macrophage cells, shedding light on the immunomodulatory potential of seaweeds (Fajriah et al., 2020).These references collectively demonstrate the importance of nitric oxide in the antioxidant and immunomodulatory effects of seaweeds, highlighting their potential therapeutic applications in combating inflammation and modulating immune

responses. Nitric oxide scavenging by seaweeds varies significantly among species and extraction methods, highlighting their potential as natural antioxidants. Brown seaweeds such as *cystoseira hakodatensis*, have demonstrated the highest nitric oxide scavenging efficacy, particularly in methanol extracts, attributed to their rich phenolic content and the presence of fucoxanthin, a potent carotenoid. Other brown seaweeds like *Fucus vesiculosus* also exhibit moderate nitric oxide scavenging abilities due to their alginates and phlorotannins. In contrast, red seaweeds such as *Chondrus armatus* show significant nitric oxide scavenging activity comparable to ascorbic acid, largely due to its carrageenan content, which interacts with free radicals. *Gracilaria edulis*, known for its polyphenolic compounds, also demonstrates promising nitric oxide scavenging capabilities, while green seaweeds like *ulva lactuca* have antioxidant properties, they are less studied. The method of extraction plays a crucial role in determining the antioxidant capacity, Methanol and ethanol extraction yields higher concentration of bioactive compounds compared to hot water extraction. Overall, brown seaweeds tend to outperform red and green varieties in Nitric oxide scavenging efficacy, suggesting that further research in to specific extraction techniques and the synergetic effects of various compounds could enhance the application of these marine organiisms in food preservation, nutraceuticals and therapeutic contexts(Davydova et al., 2020).

5.4.4 Assay for scavenging lipid peroxidation

The process of oxidation degradation of lipids was studied using methanol and chloroform extracts of *Nitophyllum marginale*. The results revealed that the methanol extract exhibited a lipid oxidation degradation of 69.34%, while the chloroform extract showed a slightly lower degradation of 27.54%. These findings suggest that the methanol extract has a higher capacity to inhibit lipid oxidation compared to the chloroform extract, as indicated by the higher percentage of degradation observed. The different concentrations of extracts used in the experiment likely influenced the extent of lipid oxidation degradation (shown in Table 15 & Fig 18).

Table 15: Lipid peroxidation scavenging potential of *N.marginale*

Conc. (mg/ml)	Ascorbic acid	Chloroform extract of *N.marginale*	Methanol extract of *N.marginale*
0	0	0	0
0.2	27.73±0.35	5.39±0.488	10.13±0.48
0.4	42.45±1.234	9.78±1.49	23.17±0.74
0.6	58.93±0.618	16.36±0.488	41.96±0.97

0.8	79.8±0.714	21.35±0.28	59.75±0.48
1	89.318±0.062	27.54±0.28	69.34±0.59

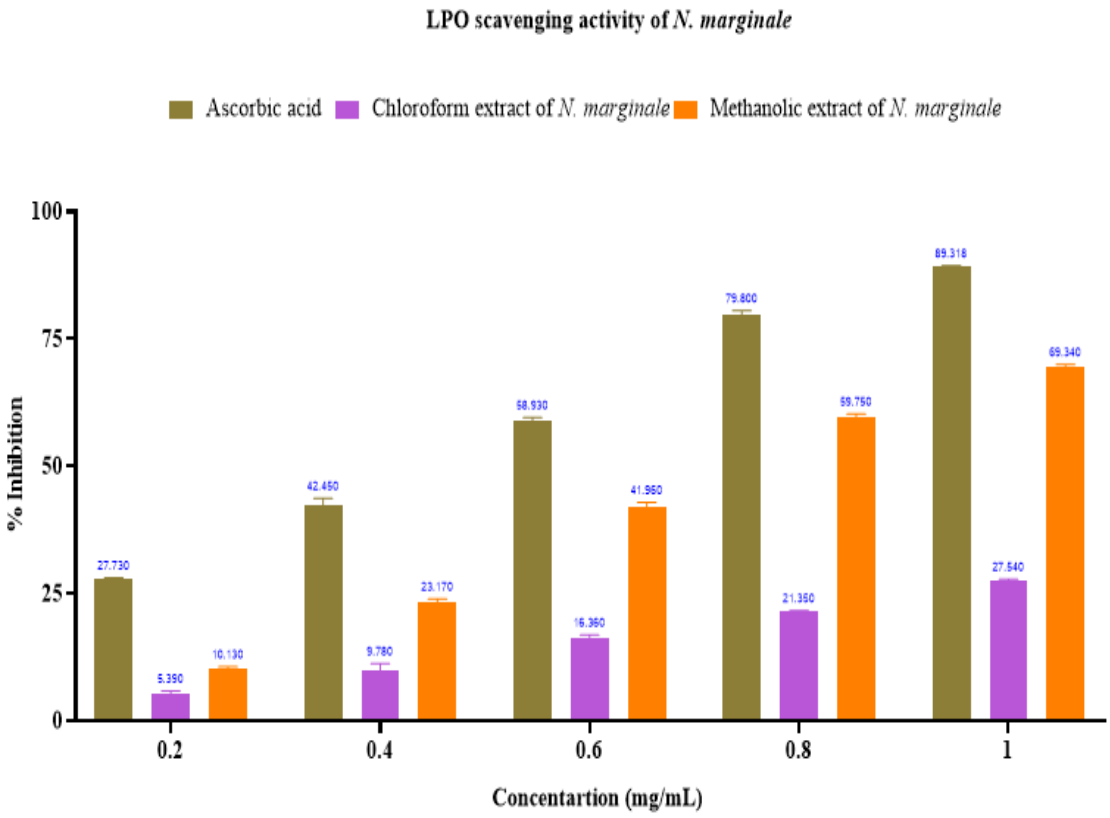


Fig 18: Graphical representation of Lipid peroxidation activity using Chloroform and methanol extract of *Nitophyllum marginale*

Seaweeds contain a wide range of bioactive lipids, including polyunsaturated fatty acids (PUFAs), which have antioxidant properties. The lipid profiles of five commonly consumed Japanese dietary seaweeds were investigated, and it was discovered that kombu has a high health promotion index and an optimal P:S ratio, indicating that it is a nutritionally important dietary seaweed (Gowda et al., 2022). Another study analysed the total lipid content and lipidic profile of seaweeds harvested in the North Coast and purchased in Portugal, finding that they contain bioactive lipids with potential health advantages. (Soares et al., 2021). Seaweed lipid extracts, fractions, and complex lipids have shown promise as functional components in the food and feed, cosmetic, and pharmaceutical industries (Lopes et al., 2021). The edible seaweed, Laminaria japonica, contains cholesterol mimics that inhibit lipid peroxidation and cyclooxygenase enzymes, demonstrating high anti-inflammatory and

antioxidant potential. (Lu et al., 2022). A study found that the methanolic fraction of seaweeds (100 μg/ml) showed lesser lipid peroxidation than the normal BHT (20-100 μg/ml)(Devi et al., 2008b). As a result, seaweeds have significant potential as antioxidants and health-promoting meals for human consumption because of their high antioxidant lipid content. Lipid peroxidation is a significant oxidative process that can lead to cellular damage and various seaweeds exhibit antioxidant properties that help mitigate this effect. Studies have shown that extracts from different seaweed species can effectively inhibit lipid peroxidation, with brown algae like *sargassum species* demonstrating the highest antioxidant activity. For instance, a study evaluated 17 seaweed species and found that *sargassum* extract significantly reduced lipoxygenase activity and showed strong radical scavenging capabilities in DPPH assays, indicating their potential as effective antioxidants against lipid peroxidation. Additionally, the complex lipids found in seaweeds, including phospholipids and glycolipids, have been recognised for their bioactive properties. These lipids not only contribute to the overall antioxidant capacity but also provide essential omega-3 fatty acids, which are beneficial for health and further enhance the protective effects against oxidative stress. Furthermore, specific brown seaweeds such as *cystoseira hakodatensis* have been highlighted for their high phenolic content and fucoxanthin levels, which synergistically enhance antioxidant activity and lipid protection. The variability in lipid composition among seaweed species also plays a crucial role in their antioxidant efficacy, *Laminaria digitata* has been shown to reduce lipid oxidation in meat products, suggesting practical application in food preservation. Overall, the diverse biochemical profiles of seaweeds present promising avenues for developing natural antioxidants to combat lipid peroxidation in various industries.(Lopes et al., 2021)

5.4.5 Assay for scavenging hydrogen peroxide

The activity of scavenging hydrogen peroxide was evaluated using methanol along with chloroform extracts of *Nitophyllum marginale*. The results indicated that the methanol extract exhibited an activity of 62.86% in scavenging hydrogen peroxide, while the chloroform extract showed a slightly lower activity of 26.94%. These findings suggest that both extracts of *Nitophyllum marginale* possess the ability to scavenge hydrogen peroxide to a certain extent, with the methanol extract displaying a slightly higher activity compared to the chloroform extract across the range of concentrations tested. The different concentrations of extracts used in the experiment likely influenced the scavenging activity observed (shown in Table 16 & Fig. 19).

Table 16: Hydrogen scavenging activity of *N.marginale*

Conc. (mg/ml)	Ascorbic acid	Chloroform extract of *N.marginale*	Methanol extract of *N.marginale*
0	0	0	0
0.2	34.47±0.47	9.57±0.27	11.7±0.37
0.4	69.74±0.23	12.13±0.54	23.15±0.29
0.6	78.159±0.116	18.3±0.27	36.47±0.28
0.8	86.39±0.134	22.6±0.47	48.11±0.46
1	95.69±0.0116	26.94±0.37	62.86±0.46

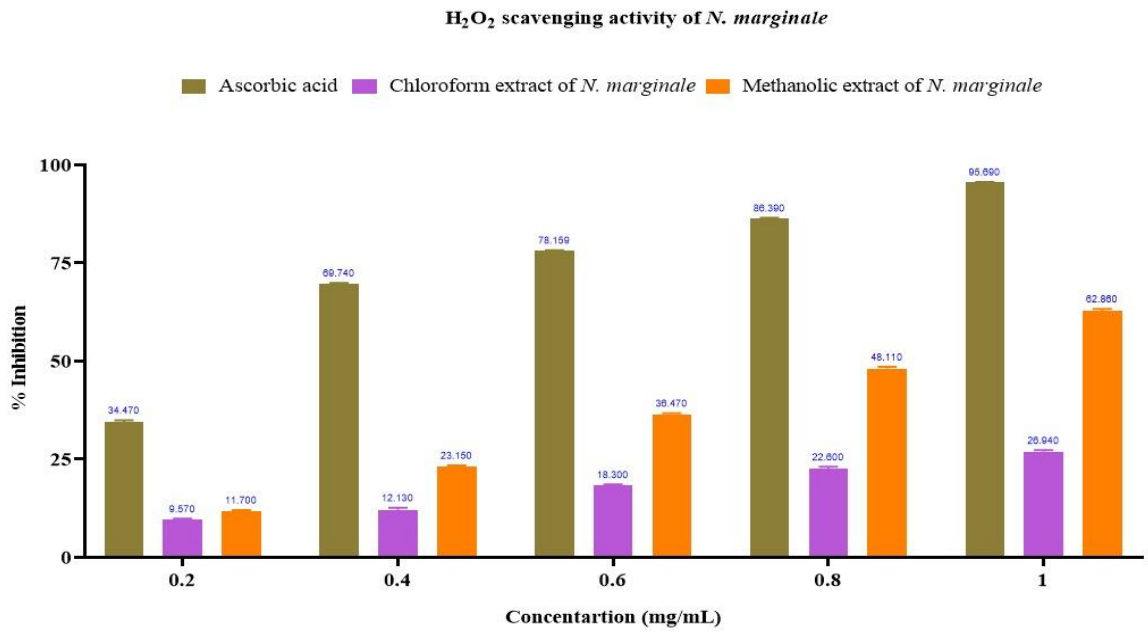


Fig 19: Graphical representation of hydrogen peroxide using Chloroform and methanol extract of *Nitophyllum marginale*

The use of hydrogen peroxide (H2O2) in seaweed aquaculture has been explored as a potential health indicator and stress bio indicator for seaweeds. Studies have shown that monitoring H_2O_2 levels in seawater could serve as an early warning sign of stress in seaweed aquaculture (Taenzer et al., 2024a). Additionally, H_2O_2 has been investigated for its strong oxidation ability and potential in gaseous mercury capture using seaweed biochars (Wei Yang et.al.,, 2023). Furthermore, the assessment of H2O2 as a bio indicator of stress in seaweed aquaculture has revealed its role as a reactive oxygen species (ROS) that could be used as an indicator of health in seaweeds, especially in response to acute stressors (Taenzer et al., 2024b). Moreover, a method for treating seaweed with hydrogen peroxide has been patented, highlighting its application in preparing seaweed powder (Peroxides & Marquis, 1978). These references collectively demonstrate the significance of hydrogen peroxide in seaweed aquaculture, both as a stress indicator and a treatment method, showcasing its potential

impact on seaweed health and cultivation practices. Hydrogen peroxide scavenging by seaweeds is an important area of research due to its implications for oxidative stress management. Various studies have demonstrated that different seaweed species exhibit significant antioxidant properties against hydrogen peroxide. For instance, *Dictyota dichtoma* showed a high percentage of hydrogen peroxide scavenging activity (over 45%) in acetone and ethanol extracts, indicating its potential as a natural antioxidant. similarly, *Turbinaria ornata* also displayed notable scavenging capabilities, while *Enteromorpha intestinalis* exhibited lower inhibition rates, highlighting variability among species. The antioxidant activity in seaweeds is often attributed to their rich phytochemical content including phenolic compounds and polysaccharides. These compounds can effectively neutralize reactive oxygen species, including hydrogen peroxide there by preventing oxidative damage. Additionally, the ability of seaweeds to produce antioxidant enzymes further contributes to their capacity to scavenge hydrogen peroxide and maintain cellular redox balance. In comparative studies, brown seaweeds generally demonstrate superior hydrogen peroxide scavenging abilities compared to red and green seaweeds, extracts from *sargassum species* have been noted for their high antioxidant activity, effectively inhibiting lipid peroxidation and radical formation. The mechanism behind these effects include electron donation and the formation of stable products that prevent further radical reactions. Overall, the diverse biochemical profiles of seaweeds present promising opportunities for developing natural antioxidants that can be utilized in food preservation and therapeutic applications against oxidative stress. Further research in to specific extraction methods and the synergistic effects of various compounds could enhance their antioxidant potential against hydrogen peroxide(Parthiban et al., 2013).

5.5 Inhibitory activity using chloroform and methanol extract of *N.marginale*

5.5.1 Alpha-glucosidase inhibition assay

The activity of inhibition against α-glucosidase enzyme were investigated with respect to methanol as well as chloroform extracts obtained from *Nitophyllum marginale*. The results demonstrated that the methanol extract exhibited an inhibitory activity of 57.93% against α-glucosidase, while the chloroform extract displayed a slightly lower activity of 32.43% with standard Acarbose at higher conc.1mg/ml showed 82.23%. These findings suggest that both extracts of *Nitophyllum marginale* possess the ability to inhibit α-glucosidase enzyme, with the methanol extract showing a higher inhibitory potential compared to the chloroform extract across the tested range of concentrations (shown in Fig.20). The observed differences

in inhibitory activity could be attributed to differences in the chemical composition and concentrations of bioactive Compounds found in the extracts. Further investigations are necessary to determine and isolate the specific compounds responsible for the α-glucosidase inhibitory activity of *Nitophyllum marginale* extracts.

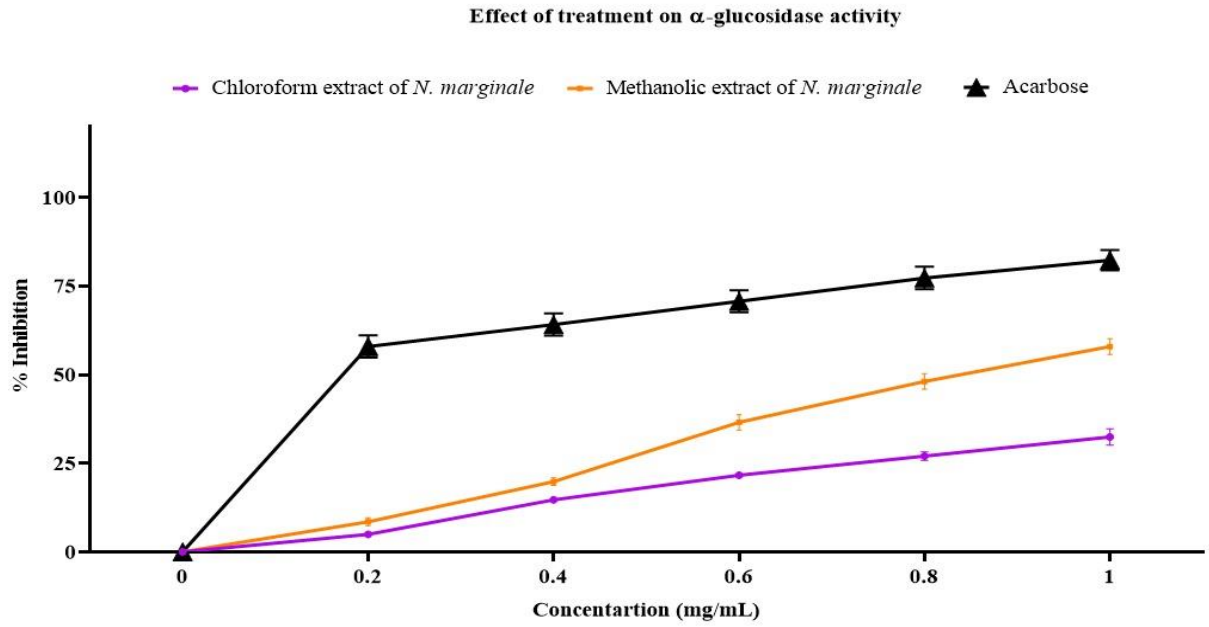


Fig 20: Graphical representation of Alpha-glucosidase using Chloroform and methanol extract of *Nitophyllum marginale*

Seaweeds have been researched for their ability to act as alpha-glucosidase inhibitors, which can aid with blood sugar regulation by slowing carbohydrate digestion. The aqueous extracts of four seaweeds collected from Gulf of Mannar coastal waters were tested for alpha-amylase and alpha-glucosidase inhibition. The results showed that the extracts of Turbinaria decurrens and Sargassum acinarium had the highest inhibitory activity against alpha-glucosidase, with IC50 values of 1.19 mg/mL and 1.21 mg/mL, respectively (G. A. Ismail et al., 2020) . Sargassum glaucescens methanolic extract inhibited alpha-glucosidase with an IC50 value of 8.90 ± 2.40 mg/mL, above acarbose's inhibition of 6.60 ± 2.10 mg/mL.(Gazali et al., 2023).The aqueous extract of the green seaweed *Halimeda tuna* showed inhibitory activity against alpha-glucosidase, with an IC50 value of 4.34 ± 0.32 mg/mL, which was higher than that of acarbose at the same concentration(Gazali et al., 2023)

Sargassum asperum's methanolic extract inhibited alpha-glucosidase with an IC50 value of 61 µg/mL, equivalent to acarbose's (57 µg/mL) (DIHARMI et al., 2023). These investigations indicate that seaweeds have the potential to be employed as alpha-glucosidase inhibitors,

which could aid in diabetes treatment. However, additional research is needed to isolate and identify the active molecules responsible for the inhibitory activity, as well as to evaluate their safety and efficacy in vivo.

One notable study is titled “Alpha-amylase and alpha-glucosidase inhibition effects of Korean edible seaweeds which aimed to evaluate the anti-diabetic activities of various Korean seaweeds against these enzymes. The research found that while many brown seaweeds showed weak inhibitory effects (IC50 values >500µg/ml) specific species like *P.arborescens* and *R.okamurae* exhibited significant inhibition with IC50 values of 260µg/ml and 50.63µg/ml respectively. Notably, the green seaweeds *C.wrightiana var. minor* demonstrated a strong alpha-glucosidase inhibition indicating its potential as a natural antidiabetic agent. The study emphasizes the need for further investigation in to effective diabetes management solutions, and also evaluating their inhibitory effect on two key enzymes involved in carbohydrate metabolism. The research utilized ethanolic extracts from a range of brown, green and red seaweeds compairing their efficacy against commercial inhibitors like acarbose. The study emphasizes the need for further exploration of the bioactive compounds with in these seaweeds to develop effective natural therapies for diabetes. Several studies have explored the inhibition of alpha-glucosidase, a key enzyme in glucose metabolism, highlighting its importance in diabetes management. One significant study “Discovery of new alpha glucosidase inhibitors: structural base virtual screening and biological evaluation (Ye et. al., 2019) identify four promising compounds that exhibited over 50 % inhibition at 100µm, kinetic analysis revealed these compounds acted as non-competitive inhibitors with ki values ranges from 11.27 to 24.18µm, suggesting their potential for therapeutic development against type 2 diabetes. Another study by yama moto et.al focused on synthesizing novel derivatives, particularly compounds which demonstrated strong α-glucosidase inhibition with an IC50 of 52.2µM- 14.5 times more effective than acarbose (IC50= 750µM). The study employed docking and molecular dynamics simulations to elucidate the binding interaction at the enzyme active site, confirming compounds competitive enzyme inhibition mechanism. Additionally, research on fucoidan extracted from *Undaria pinnatifila* found it to be a competitive inhibitor of α-glucosidase, exhibiting stronger inhibition than α-amylase. The study utilized Lineweaver Burk plots to demonstrate that fucoidan significantly decreased the Km value while maintaining a constant Vmax . These studies collectively underscores the significance of alpha-glucosidase inhibitors in managing postprandial blood glucose levels and highlight the ongoing search for effective and safer alternatives to traditional antidiabetic medications.(G. A. Ismail et al., 2020)

5.5.2 Alpha-amylase inhibition assay

The activity of inhibition against α-amylase enzyme was investigated using various concentrations ranging from 200-1000µg/ml of methanol as well as chloroform extracts obtained from *Nitophyllum marginale*. The results demonstrated that the methanol extract exhibited an inhibitory activity of 43.85 % against α-amylase, while the chloroform extract displayed a slightly lower activity of 38.66%. These findings suggest that both extracts of *Nitophyllum marginale* possess the ability to inhibit α-amylase enzyme, with the methanol extract showing a higher inhibitory potential compared to the chloroform extract across the tested range of concentrations

Table 17a: Alpha amylase activity using standard

Standard	Concentration (µg/ml)	OD at 540 nm	% Inhibition
Blank	**-**	0.998	-
Control	**-**	0.034	-
Acarbose	**6.25**	0.911	9.02
	12.5	0.852	15.15
	25	0.639	37.24
	50	0.346	67.63
	100	0.139	89.11

Table 17b: Alpha-amylase activity with chloroform and methanol extract of *Nitophyllum marginale*

Sample code	Concentration (µg/ml)	OD at 540 nm	% Inhibition
Blank	-	1.485	-
Control	-	0.039	-
Methanol	200	1.304	12.52
	400	1.196	19.99
	600	1.085	27.66
	800	0.975	35.27
	1000	0.851	43.85
Chloroform	200	1.349	9.41
	400	1.261	15.49
	600	1.136	24.14
	800	1.026	31.74
	1000	0.926	38.66

Seaweeds have been shown to contain alpha-amylase inhibitory properties, which can help manage blood sugar levels by slowing carbohydrate digestion. A research of four seaweeds obtained from Gulf of Mannar coastal waters indicated that the aqueous extracts of Chlorodesmis and C. aerea demonstrated the strongest inhibition against alpha-amylase, with IC50 values of 147.6 and 408.9 µg/ml, respectively (Lordan et al., 2013). Another study of 15

seaweeds indicated that the cold water and ethanol extracts of Ascophyllum nodosum had the highest alpha-amylase inhibitory activity, with IC50 values of 0.02 and 0.13 mg/ml, respectively (Unnikrishnan et al., 2015). Another investigation on 15 seaweeds indicated that the cold water and ethanol extracts of Ascophyllum nodosum have the highest alpha-amylase inhibitory activity, with IC50 values of 0.02 and 0.13 mg/ml, respectively.

The study evaluated the inhibitory effects of various seaweeds including *Helimeda tuna, Turbinaria decurrens* and others highlighting that the ethyl acetate fraction of *Halimeda tuna* exhibited an IC50 of 0.88mg/ml, comparable to acarbose. In contrast, *Turbinaria decurrens* demonstrated remarkable activity with 96% inhibition against alpha-amylase, attributed to its high phenolic content. Another research focused on brown seaweeds like *Ascophyllum nodosum* and *Fucus vesiculosus* which showed varying inhibitory effects with IC50 values ranging from 0.26 to 0.47mg/ml. The study emphasized that brown seaweeds generally possess stronger inhibitory effects compared to green and red seaweeds. Further analysis of seaweed extracts from Tamil Nadu indicated that compounds such as polyphenols and fucoidan are crucial for their antidiabetic properties, influencing their effectiveness in inhibitory α-amylase. These findings suggest that specific species of seaweeds from Tamil Nadu could be valuable in developing natural inhibitors for managing diabetes, further investigation in to their bioactive compounds and mechanism of action(Motshakeri et al., 2014).

5.6 Cytotoxicity assay

5.6.1 MTT assay:

The cytotoxicity effects observed in 3T3 cell lines were slightly influenced by the varying concentrations of chloroform and methanol extracts. High cytotoxicity was observed in the tested samples at higher concentrations after 48 hours of treatment in the 3T3 cell line. Additionally, it was found that increasing the concentration of the test samples resulted in higher cytotoxicity against the 3T3 cell line. The IC50 concentrations for the chloroform and methanol extracts were determined to be 192.80 µg/ml and 185.42 µg/ml, respectively, against the 3T3 cell line (Fig.21).

Table 18: *In vitro* cytotoxicity effect of samples chloroform and methanol extract against 3T3 cell line.

Concentrations (ug/ml)	Cell viability (%)	
	Chloroform extract	Methanol extract

0.0	100.00	100.00
12.5	97.06	96.67
25.0	80.57	86.68
50.0	74.70	77.04
100.0	70.54	69.95
200.0	51.04	48.69

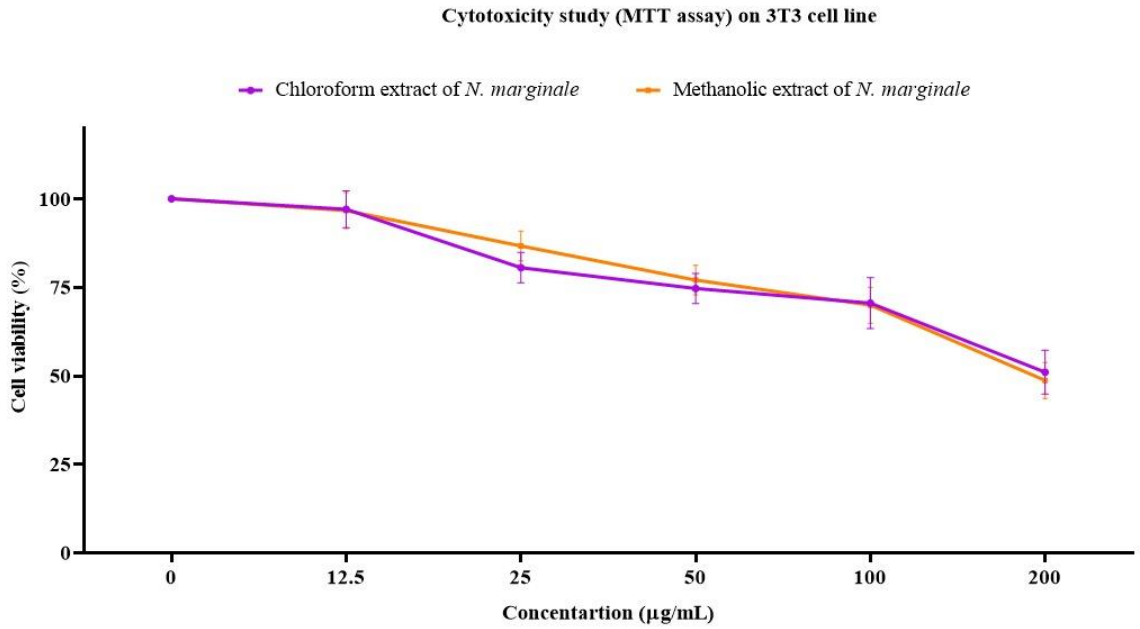


Fig 21: Graphical representation of cytotoxicity assay with chloroform and methanol extract of *Nitophyllum marginale*

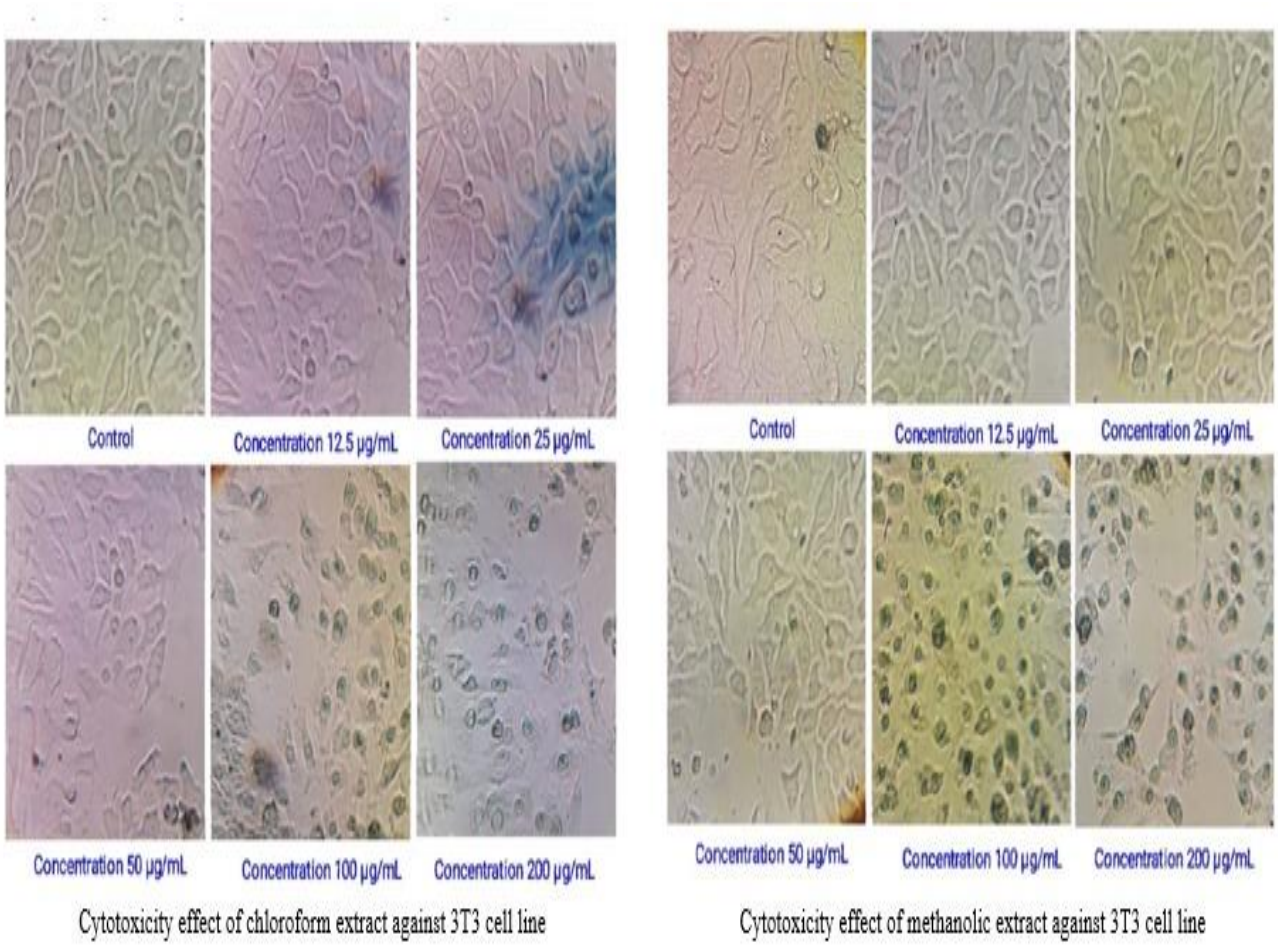


Fig 22: Cytotoxicity effect of chloroform and methanol extract against 3T3 cell line.

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay is a widely used method to assess cell viability and cytotoxicity in various research studies. In the context of seaweed research, the MTT assay has been employed to evaluate the cytotoxic properties, antitumor effects, and potential health benefits of seaweed extracts. A study focused on the cytotoxicity assay of Fucoidan extracted from *Turbinaria conoides* using the MTT assay to quantify its cytotoxic properties(Santhanam et al., 2022). This research aimed to understand the impact of Fucoidan on cell viability, providing valuable insights into the potential health implications of this seaweed-derived compound.

Another study screened for the in vitro cytotoxic activity of seaweed, *Sargassum sp.,* against various cell lines using the MTT assay. This research documented the percentage of cell viability and explored the cytotoxic effects of seaweed extracts, shedding light on their potential applications in health and medicine (J Stella Mary 1, P Vinotha, 2012). Preliminary screening of aqueous extracts from different seaweeds involved conducting the MTT assay to

assess cytotoxicity effects against mouse fibroblast cell lines. This study aimed to evaluate the safety profile of seaweed extracts and their impact on cell viability, contributing to the understanding of the biological activities of seaweed compounds(Premarathna et al., 2020).

These references highlight the significance of the MTT assay in seaweed research, providing valuable data on cytotoxicity, antitumor effects, and potential health benefits associated with seaweed extracts. The MTT assay serves as a crucial tool in evaluating the biological activities of seaweed compounds and their implications for various applications in the fields of nutrition, medicine, and biotechnology.

The cytotoxicity and bioactive potential of various seaweeds from Tamil Nadu have gathered significant attention, particularly regarding their effects on different cell lines, including the 3T3-L1 preadipocyte cell line. Seaweeds are rich in bioactive compounds such as polysaccharides, unsaturated fatty acids, phenols and peptides, which contribute to their therapeutic properties against diseases like cancer and diabetes. For instance, the red algae *Gracilaria corticata* collected from the mandapam coast of Tamil Nadu, has been studied for its antibacterial, antioxidant and anti-cancer potentials. In a study by jayasree et. al. the methanolic extract of *G.corticata* was shown to exhibit significant cytotoxicity against the MDA-MB 231 human breast cancer cell line using the MTT assay, indicating its potential as an anticancer agent. The antioxidant activity was assessed using the DPPH radical scavenging assay, revealing a strong capacity to inhibit oxidative stress. Another study focused on *Kappaphycus alvarezii*, known for its high nutritional value and bioactive compounds. The species demonstrated notable anti-microbial activity against various pathogenic bacteria, which was attributed to its rich phytochemical profile, including flavonoids and phenolic compounds. The presence of these compounds not only enhances its medicinal properties but also suggests potential applications in food preservation and functional food development. Moreover, *cymodocea serrulata*, while primarily a seagrass, has been analyzed alongside seaweeds for its bioactive constituents. It contains significant levels of phenolic compounds and other secondary metabolites that contribute to its anti-microbial and anti-oxidant activities. The comparative analysis of these seaweeds highlights their diverse biological activities and supports their use as natural sources of therapeutic agents. Overall, the findings from studies involving seaweeds in Tamil Nadu underscore their potential as valuable resources for developing neutraceuticals and pharmaceuticals aimed to combating various health issues. The ongoing research in to their bioactive compounds could

lead to innovative solutions in cancer therapy and metabolic disease management(Of et al., 2014).

5.7 Glucose uptake assay

The glucose uptake assay using methanolic and chloroformic extracts of *Nitophyllum marginale* on L6 cells revealed a significant increase in glucose uptake compared to control, both extracts demonstrated comparable or superior effects to insulin as a positive control.

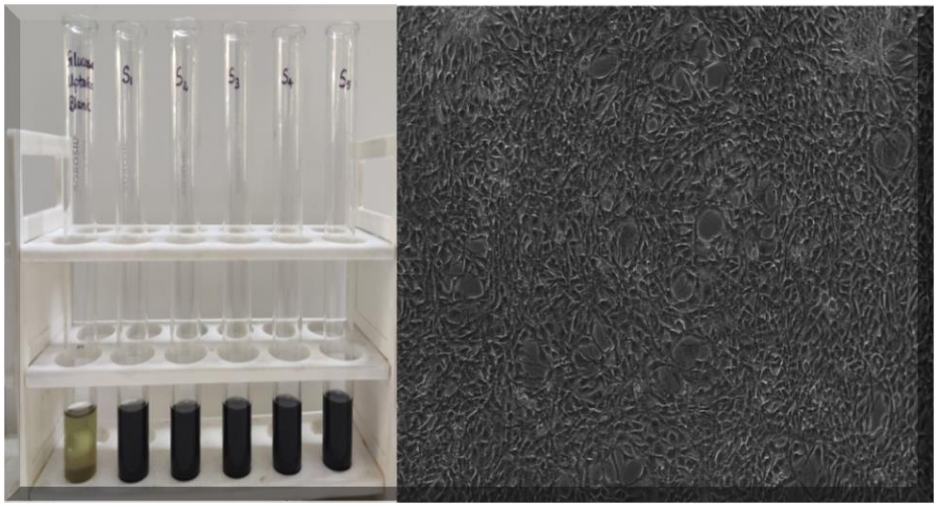


Fig 23: Glucose uptake assay using Chloroform and methanol extract of *Nitophyllum marginale* effect on L6 cellline (rat skeletal muscle)

The dose response relationship indicated a dose dependent increase in glucose uptake, with a potential saturation point at higher concentrations (shown in Table 18)

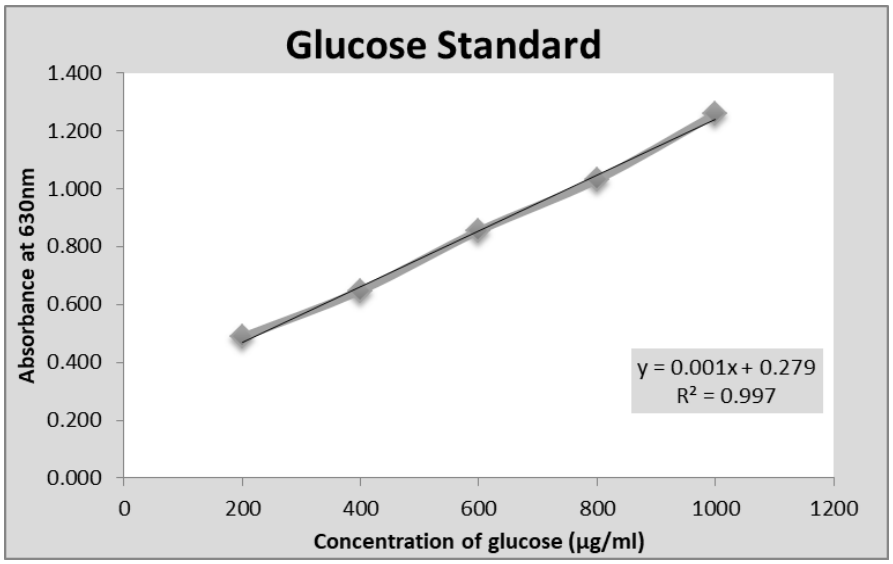


Fig 24: Glucose standard graph

These findings suggest that *N.marginale* extracts contain bioactive compounds that positively modulate glucose metabolism, holding promise for diabetes treatment pending further in vivo validation & exploration of specific compounds responsible for these effects.

Table 19: Effect of extract of *N.marginale* on glucose uptake in L-6 cell line.

Sample conc.	Absorbance at 630nm				Glucose conc. (ug/ml)	Percentage Glucose uptake
	Triplicate1	Triplicate2	Triplicate3	Average		
Control (untreated)	0.968	0.962	0.966	0.965	686.33	31.37
25ug/ml	0.706	0.703	0.703	0.704	425.00	57.50
50ug/ml	0.598	0.594	0.596	0.596	290.00	71..30
100ug/ml	0.392	0.397	0.394	0.394	115.33	93.47
Methanol						
25ug/ml	0.944	0.949	0.941	0.945	665.67	37.43
50ug/ml	0.918	0.911	0.913	0.914	635.00	46.12
100ug/ml	0.868	0.873	0.872	0.871	592.00	58.89
Chloroform						
25ug/ml	0.953	0.954	0.952	0.953	674.00	22.60
50ug/ml	0.945	0.942	0.941	0.943	663.67	28.63
100ug/ml	0.938	0.932	0.939	0.936	678.33	32.27

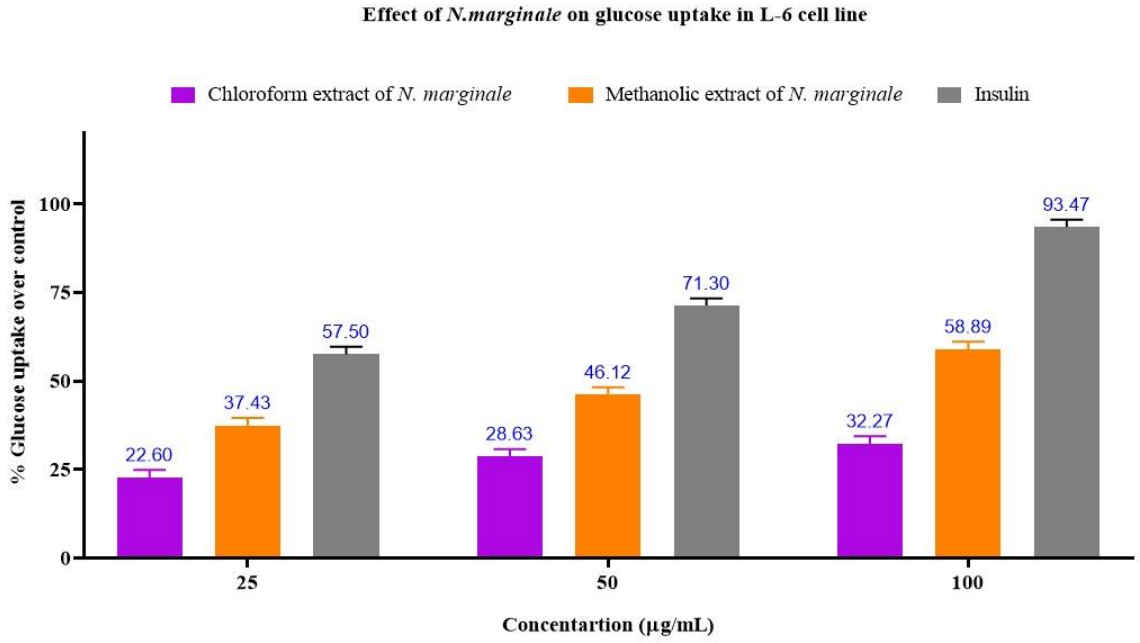


Fig 25: Glucose uptake in L-6 cell line using chloroform and methanol extract of *Nitophyllum marginale*

In vitro studies were conducted on glucose utilisation in L-6 cell lines. Figure 25 shows the results. The glucose uptake by 25 µg/ml of chloroform extract, methanolic extract and insulin showed 22.60, 37.40 and 57.50 respectively while at higher concentration i.e 100µg/ml the effect was 32.27, 58.89 and 93.47 by chloroform extract, methanol and insulin respectively. The experiment compared results with insulin, an injectable anti-diabetic medication used as the standard treatment. These measurements were taken relative to a control group. Insulin was observed to enhance glucose uptake in L6 cells in a dose-dependent manner compared to untreated cells (control). The maximum effect on glucose uptake was seen at a concentration of 100 µg/mL.

The study revealed that the treatment significantly improved glucose absorption in L6 cells compared to untreated cells. Glucose uptake increased proportionally with increasing concentration of the treatment, reaching maximum activity at 100 µg/mL.

L6 cells are a valuable model for studying glucose uptake because they are commonly used to investigate this process in muscle cells, possess an insulin signaling pathway, and express GLUT4, which is sensitive to insulin. Skeletal muscle plays a crucial role in postprandial (after-meal) glucose uptake and is the largest tissue in the body, highlighting the importance of its proper function in regulating blood glucose levels (Thiebaud et al., 1982). Impairments in insulin-stimulated glucose uptake by skeletal muscle are common pathophysiological features observed in non-insulin dependent diabetes mellitus (NIIDM). (A D Baron 1, M Laakso, G Brechtel, n.d.). GLUT4 is the primary glucose transporter present in insulin-sensitive tissues like skeletal muscle and adipose tissue. When exposed to insulin, GLUT4 quickly moves from an intracellular storage location to the cell surface membrane, aiding in glucose uptake. The results of this study indicate that the methanolic extract enhances glucose absorption in vitro. This effect might be linked to its impact on receptor levels in the skeletal muscle cell line (Suzuki & Kono, 1980).

A comparative analysis of glucose uptake using the L6 cell line has revealed significant insights in to the mechanism through which various natural extracts enhance glucose metabolism, particularly in the context of diabetes management. The L6 cell line derived from rat skeletal muscle, is widely used to study glucose transport due to its expression of GLUT4, the primary glucose transporter involved in insulin-mediated glucose uptake. One prominent study investigated the effects of ethanolic extracts from *Folium sennae*. The research demonstrated that this extract significantly increased glucose uptake in L6 cells,

achieving a 2.04-fold increase at optimal concentrations compared to control. The mechanism was elucidated through the activation of key signalling pathways, including AMPK and PKC, which facilitated GLUT4 translocation to the plasma membrane. The study also highlighted the role of intracellular calcium levels in enhancing glucose uptake as the elevation of cytosolic calcium was found to be critical for GLUT4 movement and subsequent glucose uptake. Another significant study focused on *Helicteres isora*, a traditional medicinal plant known for its antidiabetic properties. The hot water extract was shown to enhance glucose uptake by 28.99% over control at a conc. of 200µg/ml. This effect was comparable to that of insulin and metformin, indicating its potential as a natural hypoglycaemic agent. The study suggests that *Helicteres isora* activates glucose transport mechanism in skeletal muscle cells, further supporting its traditional use in diabetes treatment. Additionally, research on *Fucus racemosa* demonstrated substantial glucose uptake potential in L6 myotubes. The extract exhibited significant increases in glucose uptake when compared to metformin, a standard antidiabetic drug. This study emphasized the importance of GLUT 4 translocation in mediating glucose uptake and suggested that plant derived compounds could offer new avenues for developing effective diabetes treatment. Moreover, studies have also explored the role of α1-adrenoceptors in regulating glucose uptake in L6 cells. Activation of these receptors by agonists such as phenyl ephrine led to increased glucose uptake through pathways involving phospholipase c and protein kinase c (PKC) interact with insulin pathways to modulate glucose metabolism. In summary, the comparative studies on glucose uptake using the L6 cell line underscores the potential of various natural extracts from plants and seaweeds as effective agents for enhancing glucose metabolism and managing diabetes. These findings highlight the importance of understanding the underlying mechanism, including signalling pathways and GLUT4 translocation, which could lead to innovative therapeutic strategies for diabetes management(Zhao et al., 2018).

5.8 Animal Experimentation

For this investigation, sixty male wistar rats weighing 250-300 grammes were obtained from the National Institute of Pharmaceutical Education and Research (NIPER) located in Mohali, Punjab, India. The rats were kept in propylene cages covered with husk and maintained under regulated circumstances, including a 12-hour light/dark cycle, temperature of 25±2 oC, and relative humidity of 55±10%. During the trial, the rats were given a regular pellet diet and free access to water.

The institutional animal ethics committee (IAEC) of Lovely Institute of Technology (Pharmacy) at Lovely Professional University (LPU) in Punjab, India, authorised the experimental method with the protocol number LPU/IAEC/2023/34.

5.8.1 Acute toxicity study

5.8.1.1 General signs and behavioural analysis

Throughout the acute toxicity trial, rats were orally administered with methanol extract and did not succumb to treatment-related causes. Physical examinations conducted on all doses of methanol extract-treated rats for acute oral toxicity tests did not reveal any evidence of adverse effects such as changes in skin and fur, eyes and mucous membranes, behavior patterns, tremors, salivation, diarrhea, or coma. Additionally, none of the animals exhibited any apparent or microscopic clinical abnormalities.

According to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS), the methanol extract falls under Category 5. This classification carries significant implications for the protection of both animal and human health, particularly at the high dose level utilized in this study. Table 12 presents the effects of the methanol extract on the body weights of the control and methanol extract-treated rats. Over the course of the study, both the control and methanol extract groups demonstrated a gradual increase in body weight. Importantly, the percentage changes in body weight observed in the methanol extract-treated groups were not significantly different from those of the control rats ($p > 0.05$)

Table 19: Effect of extract on the percentage of body weight gain

Days	Control	50 mg/Kg	300 mg/Kg	2000 mg/Kg
0 (g)	242.21±6.22	248.58÷5.19	250.12÷6.28	239.43÷7.22
7 (Increase in %)	4.86÷1.83	4.39÷1.19	4.58÷1.06	4.64÷1.31
14 (Increase in %)	6.13÷1.34	5.18÷1.22	5.23÷1.17	5.31÷1.19

Table 20: Food consumption (g) of rats in acute oral toxicity tests

Days	Control	50 mg/Kg	300 mg/Kg	2000 mg/Kg
7	60.56 ± 2.80	57.49÷2.66	58.68÷2.36	58.22÷2.51
14	68.42 ± 2.73	65.33÷2.56	64.14÷2.42	66.38÷2.44

Table 21: Rats' water intake (ml) during acute oral toxicity testing

Days	Control	50 mg/Kg	300 mg/Kg	2000 mg/Kg
7	110.36 ± 1.12	107.49÷1.22	110.23÷1.16	108.02÷1.03
14	110.22 ± 0.93	107.32÷1.06	110.18÷1.14	109.01÷1.02

Table 22: Relative organ weight (g%) of rats in the acute oral toxicity investigation

Organs	Control	50 mg/Kg	300 mg/Kg	2000 mg/Kg
Heart	0.42±0.06	0.41±0.02	0.40±0.01	0.41±0.03
Liver	2.49±0.16	2.44±0.12	2.43±0.14	2.45±0.16

Kidney	0.67±0.03	0.61±0.02	0.59±0.01	0.62±0.03

Throughout the study, the methanol extract groups' food consumption was shown to be no different than the control group, with $p > 0.05$ (Table 13). In the acute oral toxicity test, there was no significant difference in water intake between the control and methanol extract groups ($p > 0.05$) (Table 15). There was no significant difference in ROW between the control and methanol extract-treated test groups ($p > 0.05$) (Table 16).

5.8.1.2 The extract's effect on serum biochemical markers during acute oral toxicity testing

Table 17 displays biochemical parameter data for the control and methanol extract-treated groups in the acute oral toxicity test. The biochemical indicators showed no significant difference between the groups ($p > 0.05$).

Table 23: Biochemical characteristics of rats in acute oral toxicity tests

Biochemical parameters	Control	50 mg/Kg	300 mg/Kg	2000 mg/Kg
Urea (mmol/L)	4.23±0.33	4.31±0.26	4.12±0.31	4.21±0.22
Creatinine (umol/L)	31.22±2.32	30.14±1.43	30.03±1.19	31.02±1.23
Albumin (g/L)	39.51±3.11	38.24±3.31	37.28±2.42	37.18±3.04
Globulin (g/L)	26.11±0.82	24.18±1.34	22.20±1.21	21.11±1.43
Total bilirubin (umol/L)	2.0±0.00	1.00±0.00	1.00±0.00	1.00±0.00
ALP (U/L)	162.27±5.12	166.12±4.02	163.19±5.12	167.34±4.26
ALT (U/L)	24.32±1.27	26.14±1.18	26.29±1.13	25.33±1.21
AST (U/L)	92.22±6.83	98.21±5.23	96.32±4.53	99.02±2.18

5.8.1.3 The impact of extract on haematological parameters in acute oral toxicity testing

There was no significant difference in haematological parameters between groups ($p > 0.05$, see Table 18). All haematological indicators, including haemoglobin (HB) and total white blood cell count, were found to be within normal ranges in both the control and methanolic extract-treated groups. The ANOVA test shows no significant correlation between the groups in both acute and sub-acute toxicity tests ($p > 0.05$).

Table 24: Hematological parameters of the rats in acute oral toxicity tests

Hematological parameters	Control	50 mg/Kg	300 mg/Kg	2000 mg/Kg
HB (g/L)	152.12±8.23	148.14±9.12	149.13±11.11	148.63±10.32
WBC (10^9/L)	7.62±0.21	7.74±0.23	7.73±0.29	7.83±0.27
Neutrophil (10^9/L)	0.57±0.11	0.56±0.11	0.58±0.12	0.58±0.10
Lymphocyte (10^9/L)	6.32±0.67	6.64±0.72	6.66±0.71	6.76±0.62
Monocyte (10^9/L)	0.15±0.03	0.14±0.03	0.13±0.02	0.13±0.03
Eosinophil (10^9/L)	0.07±0.02	0.09±0.02	0.10±0.02	0.10±0.02
Basophil (10^9/L)	0.02±0.01	0.04±0.02	0.03±0.01	0.03±0.02

The oral route of administration is the most practical and commonly utilized method for conducting toxicity studies. Although absorption may occur gradually, this approach is cost-

effective and less distressing for the animals. Prior to oral administration of crude extracts, animals are required to fast to minimize potential interference from food and other substances in the digestive system that could affect the response to the test materials. All procedures were conducted in accordance with relevant OECD guidelines (Adomi et al., 2017).

The test protocol involving a starting dose of 500 mg/kg body weight is commonly employed when there is no prior indication suggesting that the test chemical is hazardous (Guideline et al., 2001). In this study, rats in the control group received the vehicle, while rats in the methanolic extract-treated groups were administered crude extracts. The initial dose of 50 mg/kg body weight did not result in any deaths among the experimental animals. Following OECD Guidelines 423, subsequent higher doses of 300 and 2000 mg/kg body weight were selected for evaluation. Rats were monitored daily until the end of the experiment (day 14) for signs of toxicity and mortality. Clinical symptoms were closely observed as they are critical indicators of potential toxic effects on organs within the treated groups (Jothy et al., 2011). Throughout the 14-day acute toxicity assessment, all rats orally administered with methanolic extract at single doses of 50, 300, or 2000 mg/kg did not display any signs of distress, toxicity markers, or mortality. There were no observable changes in their overall well-being. Rats in the control group and those treated with methanolic extract at doses of 300 mg/kg and 2000 mg/kg exhibited normal skin, fur, eyes, mucous membranes, salivation, behavior, and sleep patterns. None of the animals exhibited symptoms such as lethargy, tremors, diarrhea, or coma. Additionally, the body weights of rats in both the control and methanolic extract-treated groups increased without significant differences noted.

Based on these findings, the investigation concluded that the methanol extract does not induce acute toxicological effects and no fatalities occurred. Following OECD guidelines (specifically OECD 423), these results allow for the substance to be categorized and classified using the Globally Harmonized System of Classification and Labelling of Chemicals. Therefore, the methanol extract can be classified as Category 5 with low acute toxicity risk, representing the lowest toxicity class (Guideline et al., 2001). As a result, it is possible to conclude that methanol extract is tolerated up to 2000 mg/kg body weight when administered as a single dose (Ramaswamy et al., 2012). Acute toxicity information has limited therapeutic applicability since cumulative toxic effects occur even at relatively low dosages.

Acute toxicity studies of seaweeds have become an essential aspect of evaluating their safety for potential therapeutic applications. Recent research has focused on the acute toxicity profile of fucoidan derived from *sargassum wightii*, a brown marine alga commonly found along the coast of Tamil Nadu. In a study conducted according to OECD guidelines, a single oral dose of fucoidan (2000mg/kg) was administered to female wistar rats. The results indicated no mortality or significant toxic signs during the observation period, which lasted for 14 days. The animal was monitored for behavioural changes, body weight and any abnormal clinical signs such as salivation, lacrimation and tremors. The findings showed that fucoidan did not induce any adverse effects, reinforcing its safety for potential medicinal use and encouraging further exploration of its therapeutic properties in managing conditions such as cancer and diabetes. Another study evaluated the acute toxicity of sulfated polysaccharides extracted from various edible seaweeds including *Porteria hornemanii* and *Asparagopsis taxiformis*. These polysaccharides were tested using L6 cell line to assess their cytotoxic effects. The results demonstrated that while some extracts exhibited cytotoxicity at higher conc., they donot show significant acute toxicity in vivo when administerd to animal models. The study highlighted that the sulfated polysaccharides had beneficial antioxidant properties, which could mitigate oxidative stress without causing harm to vital organs. Furthermore, research on *Gracilaria corticata*, another seaweed species from Tamil Nadu, indicated its potential as a safe source of bioactive compounds. In vivo studies showed that extracts from *G. corticata* did not result in any significant changes in body weight or organ morphology after administration at various doses. This suggests that *Gracilaria corticata* could be safely incorporated in to dietary supplements or functional foods aimed at promoting health benefits without posing acute toxicity risks. In summary, the acute toxicity studies conducted on various seaweed species from Tamil Nadu demonstrate their safety profiles and potential for therapeutic applications. The absence of significant toxic effects in animal model supports the continued exploration of these marine resources for developing natural products with health-promoting properties ongoing research is crucial to fully understand the long term safety and efficacy of these seaweeds in clinical settings(Manivanna et al., 2008)

5.8.2 Pharmacodynamic study

5.8.2.1 Effect of treatment on Body weight

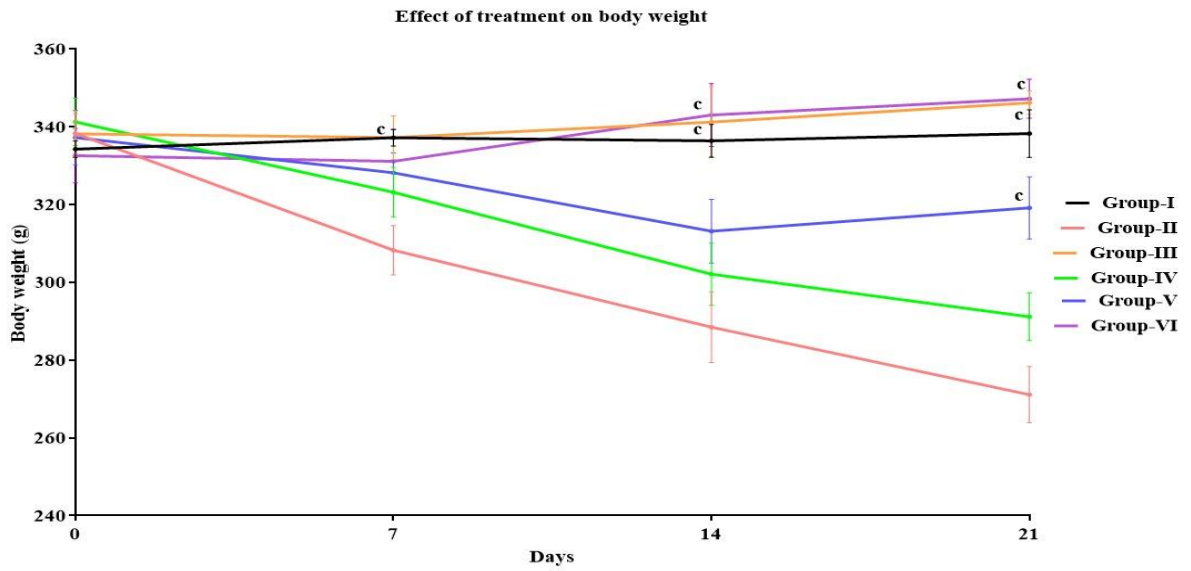


Fig.26: The effects of treatment on body weight Group I: normal control (non-diabetic), receiving normal saline, Group II: untreated diabetic control, receiving 150 mg/Kg alloxan monohydrate, Group III: diabetic rats treated by the standard diabetic drug glibenclamide (10 mg/kg), Group IV, V and VI: diabetic rats treated by methanolic extract of *N. marginale* at low, medium and high doses, respectively The values are the mean ± SEM of six rats in each group (n=6). One-way ANOVA was used in the statistical analysis. c=*p < 0.05 compared to diabetes control.

On day 0, the weight of the animal was found normal in all the group of rats as they did not received any of the inducer agent (alloxan) and drug treatment but decrease in the body weight was observed in all the group of rats received alloxan on day 7. The body weight was found to reduce 8.88, 16.48 and 21.41 percent on day 7th, 14 th and 21st respectively in group of rats received alloxan only (group II).On the other hand, the percentage reduction in the body weight was found 2.27, 5.27, 4.88 and 0.30 in group III, IV, V and VI respectively on day 7th of the experimental period. Similarly, the pattern of reduction in the body weight was observed in group II, IV, and V while improvement in the body weight was recorded in group III and VI only i.e 341.19± 9.11 and 343.00 ± 8.06 respectively on day 14th of the experimental duration. It is very important to note that the amelioration in the reduction of the body weight was observed in all the groups except group II (271.11± 7.23) and IV (291.12 ± 6.13).

Hence from this it is appear that high and medium dose is able to protect the reduction in body weight because of diabetes even better than standard drug. The reason behind such observation might be due to its better antioxidant as well as nutritive potential.

The comparative effects of seaweed treatments on body weight in alloxan-induced diabetic models have shown promising results, indicating the potential of marine algae as natural

therapeutic agents for managing diabetes. Alloxan, a compound that selectively destroys insulin-producing beta cells in the pancreas, induces hyperglycemia and weight loss in experimental animals, mimicking type 1 diabetes. Recent studies have explored the impact of various seaweed extract on body weight recovery and metabolic parameters in alloxan induced diabetic rats. One study investigated the effects of *Turbinaria decurrens* acetone extract on diabetic rats, the treatment demonstrated significant antihyperglycemic activity, with a marked reduction in elevated blood glucose levels. Rats treated with *T.decurrens* at doses of 150mg/kg and 300mg/kg showed a notable recovery in body weight compared to untreated diabetic controls. Additionally, the extract improved liver and kidney function along with total protein and hemoglobin levels, suggesting its multifaceted role in mitigating diabetes related complications. The presence of bioactive compounds such as phenols and flavonoids in *T.decurrens* was attributed to these beneficial effects, enhancing antioxidant activity and reducing oxidative stress associated with diabetes. Another significant study focused on *Sargassum polycystum*, which was administered to diabetic rats at doses of 150mg/kg. The results demonstrated that the seaweed extract not only reduced hyperglycemia but also alleviated liver and kidney damage associated with diabetes. Histopathological examination revealed improvements in pancreatic morphology, indicating a restoration of normal function. The study concluded that *C.polycystum* could effectively enhance body weight recovery and improve overall metabolic health in diabetic rats. Furthermore research on *Ulva lactuca* highlighted its potential to reduce hyperlipidemia and improve body weight in alloxan-induced diabetic rats. The administration of this green algae extract led to significant decreases in serum cholesterol and triglyceride levels while promoting weight gain compared to untreated diabetic controls. The study emphasized the importance of dietary inclusion of seaweeds like *Ulva lactuca* in managing diabetes and its complications. In summary, the comparative analysis of various seaweeds treatment on body weight recovery in alloxan-induced diabetic model underscores their potential as natural antidiabetic agents. Seaweeds such as *Turbinaria decurrens, Sargassum polycystum* and *Ulva lactuca* have demonstrated significant effects on glycemic control, metabolic health and overall body weight recovery, supporting their use in developing functional food or supplements for diabetes management. Further research is to isolate specific bioactive compounds responsible for these beneficial effects and to explore their mechanisms of action in greater detail.(Ramu et al., 2020)

5.8.2.2 Effect on Treatment of Blood Glucose

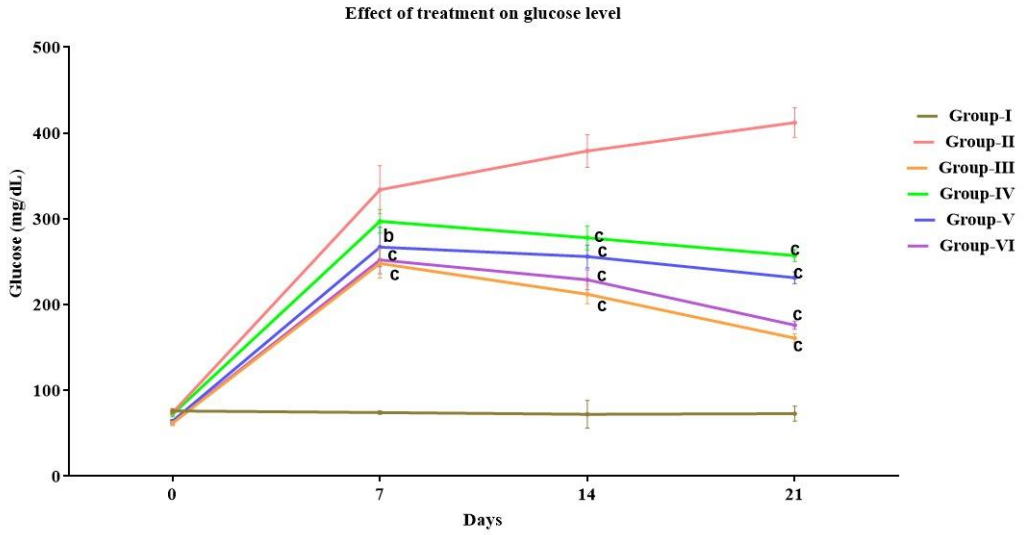


Fig.27: Effects of treatment on blood glucose, Group I: normal control (non-diabetic), receiving normal saline, Group II: untreated diabetic control, receiving 150 mg/Kg alloxan monohydrate, Group III: diabetic rats treated by the standard diabetic drug glibenclamide (10 mg/kg), Group IV, V and VI: diabetic rats treated by methanolic extract of *N. marginale* at low, medium and high doses, respectively Values represent the Mean± SEM of six rats in each group (n=6). Statistical analysis was performed using one-way analysis of variance with post-hoc testing. c=*p<0.05 compared to the diabetic control.

On day 0, the blood glucose of the animal was found normal in all the group of rats as they did not received any of the inducer agent (alloxan) and drug treatment but increase in the blood glucose was observed in all the group of rats received alloxan on 7^{th} day . The blood glucose was found to reduced on day 7^{th} , 14^{th} and 21^{st} respectively on day 7^{th} of the experimental period.Similarly, the pattern of increase in blood glucose wasobserved in group II,IV and V while improvement in the blood glucose was recorded in group III and VI only i.e 212.11± 11.23 and 229.00 ± 1.00 respectively on day 14^{th} of the experimental duration.It is very important to note that improvement in blood glucose was oserved in all the groups treated except groupII (412.33 ± 17.3) and group IV (257.12 ± 7.00).

Hence from this it is appear that high dose is able to shown slightly improvement in blood glucose with respect to standard groupIII. The reason behind such observation might be due to its better antioxidant as well as nutritive potential.

The comparative effects of seaweeds extract on blood glucose levels in alloxan-induced diabetic models have been extensively studied, highlighting the potential of these marine organisms as natural antidiabetic agents. Alloxan is known to induce diabetes by selectively damaging pancreatic beta cells, leading to hyperglycemia and associated metabolic

disturbances. One significant study investigated the effects of *Turbinaria decurrens* acetone extract on blood glucose levels in diabetic rats. The study found that administering the extract at doses of 150mgkg and 300mgkg resulted in a marked reduction in elevated blood glucose levels, demonstrating anti-hyperglycemic activity. In addition to lowering blood glucose, the treatment also improved liver and kidney function markers, along with total protein and hemoglobin levels, indicating a restoration of metabolic health in the treated rats. Histopathological examination revealed improvements in pancreatic morphology, suggesting that *Turbinaria decurrens* extract could ameliorate diabetes related damage due to its rich content of bioactive phytochemicals such as phenols and flavonoids, which are known for their antioxidant properties. Another study explored the effects of fucoidan extracted from *Lessonia trabeculata*, which was administered to alloxan-induced diabetic rats over a period of 30days. The result indicated that fucoidan treatment significantly reduced blood glucose levels while enhancing the activity of antioxidant enzymes in serum and tissues. The study emphasized the protective effects of fucoidan against oxidative stress induced by alloxan, contributing to improved glycemic control and overall metabolic health. Additionally, research on *Sargassum polycystum* demonstrated its potential to lower blood glucose levels in diabetic rats. The study revealed that both ethanolic and aqueous extracts of this brown seaweed effectively reduced hyperglycemia and improved lipid profiles. The administration of these extracts also led to significant improvements in the histopathological conditions of the liver and pancreas, indicating a protective effect against diabetes related organ damage. Furthermore, a comparative analysis involving various seaweeds including *Ulva lactuca* showed that extracts from these algae could significantly lower blood glucose levels in alloxan-induced diabetic rats. The study highlighted that the hypoglycemic effects were associated with increased insulin sensitivity and enhanced GLUT4 translocation in skeletal muscle cells. In summary, the comparative studies on seaweed extracts demonstrate their promising potential as natural antidiabetic agents capable of reducing blood glucose level in alloxan-induced diabetic models. Species such as *Turbinaria decurrens, Lessonia trabeculata and sargassum polycystum* exhibit significant antihyperglycemic effects through various mechanisms, including antioxidant activity and improvement in pancreatic function. These findings support further exploration of seaweeds as functional foods or supplements for managing diabetes and complications (Abdel-Karim et al., 2022)

5.8.2.3 Effect of treatment on Liver function test

Table 25: Effect of treatment on Liver function test

Parameters (U/dl)	Group-I	Group-II	Group-III	Group-IV	Group-V	Group-VI
SGOT	42.13±2.29	84.23±5.00	43.18±2.11^{c}	60.16±3.63^{c}	53.10±1.96^{c}	47.19±1.19^{c}
SGPT	58.21±5.00	96.17±2.16	60.12±4.31^{C}	79.15±3.23^{c}	73.00±2.62^{c}	69.11±3.12^{c}
ALP	48.18±2.12	81.00±3.89	56.15±2.00^{c}	74.12±2.00^{c}	67.21±3.00^{c}	57.00±3.48^{c}

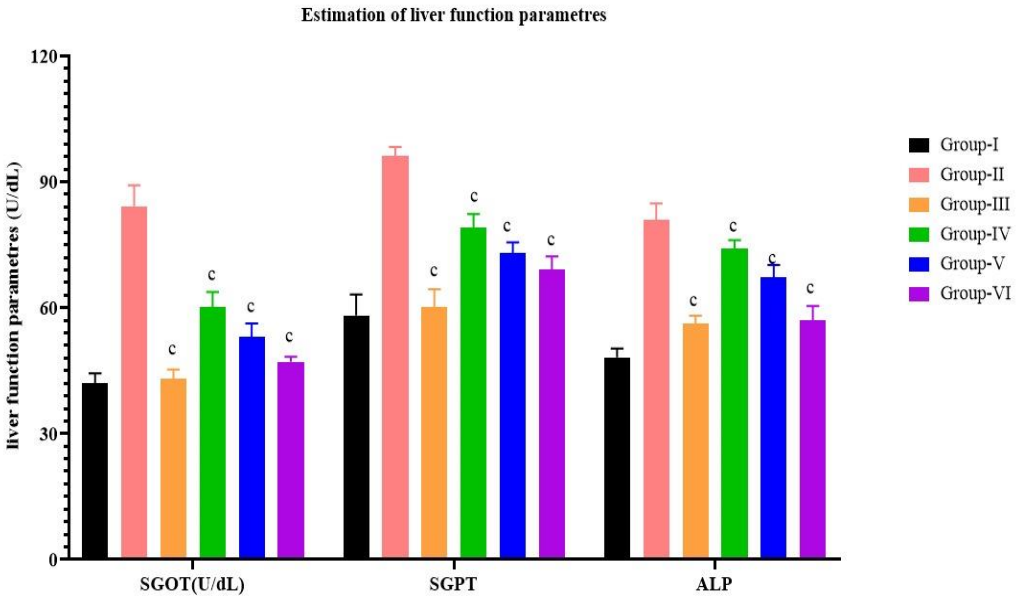


Fig.28: Effects of treatment on Liver function test, Group I: normal control (non-diabetic), receiving normal saline, Group II: untreated diabetic control, receiving 150 mg/Kg alloxan monohydrate, Group III: diabetic rats treated by the standard diabetic drug glibenclamide (10 mg/kg), Group IV, V and VI: diabetic rats treated by methanolic extract of *N. marginale* at low, medium and high doses, respectivelyValues represent the Mean± SEM of six rats in each group (n=6). Statistical analysis was performed using one-way analysis of variance with post-hoc testing. c=*p<0.05 compared to the diabetic control.

The liver enzymes were estimated in rats of group received test drug, standard, normal as well as in alloxan induced groups. The level of SGOT was found as (42.13± 2.29u/dl, 84.23± 5.00u/dl, 43.18 ± 2.11u/dl, 60.16± 3.63u/dl, 53.10± 3.18u/dl, 47.19±1.19u/dl) in Grp I, II, III ,IV, V, and GrpVI respectively. The investigation clearly indicated that Grp II showed higher level of SGOT while protection was observed in dose dependent mannar as with the highest dose the protection level was found closer to the normal group of animal even the lower dose also showed protection against elevated levels of SGOT. The findings suggested that the extract ameliorate the alloxan induced liver enzyme damage (Mallhi et al., 2023).

The liver enzyme levels were assessed in rats from different groups, including those receiving a test drug, standard, normal, and in alloxan-induced groups. The SGPT levels were measured as follows: 58.21± 5.00 u/dl (Group I), 96.17±2.16u/dl (Group II), 60.12 ± 4.31u/dl (Group III), 95.15±3.23u/dl (Group IV), 93.00 ± 2.62 u/dl (Group V), and 69.11±3.12 u/dl (Group VI).The results indicated that Group II exhibited a higher SGPT level, while a dose-

dependent protective effect was observed with the test drug. The highest dose demonstrated a protective effect similar to the normal group, and even the lower dose displayed protection against elevated SGPT levels. Overall, the findings suggest that the Methanolic extract mitigates alloxan-induced liver enzyme damage, particularly in terms of SGPT levels.

The liver enzyme levels were evaluated in rats across various groups, encompassing those administered a test drug, standard treatment, normal treatment, and in alloxan-induced groups. The ALP levels were determined as follows: 48.18±2.12 u/dl (Group I), 81.00±3.89 u/dl (Group II), 56.15± 2.00u/dl (Group III), 74.12±2.00u/dl (Group IV), 67.21±3.00 u/dl (Group V), and 57.0 ± 3.48 u/dl (Group VI). The results revealed that Group II exhibited a higher ALP level, whereas a dose-dependent protective effect was observed with the test drug. The highest dose exhibited a protective effect comparable to the normal group, and even the lower dose demonstrated protection against elevated ALP levels. In summary, the findings suggest that the extract ameliorates alloxan-induced liver enzyme damage, particularly in terms of ALP levels.

In a study conducted by Gometi et al. in 2014, it was reported that the increased levels of serum liver marker enzymes during alloxan administration were attributed to alloxan toxicity, leading to destructive changes in hepatocytes(GOMETI, et al., 2014). The enzymes SGOT and SGPT play a role in gluconeogenesis, and their transcription is negatively regulated by insulin. The study suggested that the elevation in these enzymes is not necessarily indicative of hepatocyte injury but rather linked to impaired insulin signaling. Aspartate aminotransferases and alanine transferases are released by hepatic and cardiac tissues, and elevated plasma concentrations of these enzymes serve as indicators of hepatic and cardiac damage, especially in diabetes-related complications(Anaduaka, et al., 2014). The observed reductions in SGOT and SGPT activities in the study were attributed to the protective effects of the extracts on hepatocellular and cardiac tissues. This implies that the extracts did not induce harmful effects on the liver tissues of treated rats. In contrast, untreated diabetic groups exhibited significant increases in enzyme activities, indicating potential damage to hepatic and cardiac tissues. The observed decrease in serum ALP activity implies the potential protective effects of the plant extracts on cellular and hepatocellular membranes. ALP serves as a biochemical marker enzyme crucial for preserving membrane integrity. An elevation in its plasma activity is indicative of cell membrane peroxidation, a phenomenon associated with diabetes mellitus(Osigwe et al., 2017). In last, Methanolic extract of

Nitophyllum marginale showed the reduction in elevated levels of liver enzymes with respect to alloxan induced diabetic untreated group.

5.8.2.4 Effect of treatment on Lipid profile

Table 26: Effects of treatment on Lipid profile test, Values represent the Mean± SEM of six rats in each group (n=6). Statistical analysis was performed using one-way analysis of variance with post-hoc testing. c=*p<0.05 compared to the diabetic control

Parameters (mg/dl)	Group-I	Group-II	Group-III	Group-IV	Group-V	Group-VI
TG	67.66±1.34	172.2±23	79.11±6.13^{c}	158.13±2.11	111.00±1.96^{c}	81.18±1.00^{c}
HDL	42.13±2.11	26.11±3.56	40.18±1.16^{C}	30.08±2.43	34.14±2.00^{c}	38.18±1.95^{c}
VLDL	19.19±0.32	38.00±2.34	19.18±1.00^{c}	31.18±1.00	26.11±0.31^{c}	21.18±0.21^{c}
LDL	39.13±1.13	85.16±3.00	33.19±1.23^{c}	59.26±1.32	49.12±1.14^{c}	40.16±1.00^{c}
Total Cholesterol	95.12±2.04	198.15±1.23	95.12±1.14^{c}	162.2±1.43	136.13±1.00^{c}	109.16±1.53^{c}

The lipid profile were estimated in rats of groups received test drug, standard, normal as well as in alloxan induced groups .The various parameters in lipid profile find like Triglycerides (TG), High density lipid (HDL), Very low density lipid (VLDL), Low density lipid (LDL), Total cholesterol (TC) in groups I,II,III,IV,V,VI respectively.

The values obtained in different groups of rats for TG was GI (67.66 ±1.34 mg/dl), GII (172.2 ± 23.00 mg/dl), GIII (79.11 ± 6.13 mg/dl), GIV (166.13 ± 2.11 mg/dl), GV (158.00 ± 1.96 mg/dl), GVI (81.18 ±1.0mg/dl). The investigation clearly indicated that grp II showed higher level of TG while protection was observed in dose dependent mannar as with the highest dose the protection level was found closer to the normal group of animal even the lower dose also showed protection against elevated levels of TG. The finding suggest that the extract ameliorate the alloxan induced effect cause the elevated values to be reduced and have the potential to work similar likevalues observed in standard group.

HDL values obtained GI (42.13 ± 2.11mg/dl), GII (26.11±3.56mg/dl), GIII (40.18±1.16 mg/dl), GIV (29.00± 2.43mg/dl), GV (31.14±2.00mg/dl), GVI (37.18±1.95mg/dl). GII showed lower levels with respect to normal group and the treatment group GVI with higher dose show levels to be increased with respect to untreated group (diabetic). GIV and GV also showed slight increased values.

VLDL values was GI (19.19 ±0.32mg/dl), GII (38.00±2.34mg/dl), GIII (19.18±1.00mg/dl), GIV (33.18±1.00mg/dl), GV (26.11±0.31mg/dl), GVI (21.18±0.21mg/dl). Increased values seen in GII with respect to normal group, while the extract treatment group with higher dose

GVI showed slightly decreased with respect to untreated group (diabetic) and also GIV and GV showed decreased values with respect to GII.

LDL values obtained were GI (39.13±1.13 mg/dl), GII (85.16 ± 3.00mg/dl), GIII (33.19 ± 1.23 mg/dl), GIV (59.26 ± 1.32 mg/dl), GV (51.12 ±1.14 mg/dl), GVI (44.16±1.00mg/dl) respectively. The investigation clearly noted that G2 showed higher level of LDL while protection was observed in dose dependent manner as with the highest dose the protection level was found closer to the normal group of animal even the lower dose also showed protection against the extract.

TC mean obtained were GI (95.12±2.04), GII (198.15 ±95.12), GIII (95.12 ± 1.14), GIV (172.2 ±1.43), GV (156.13±1.00), GVI (102.16 ± 1.53) respectively . The results indicates that Group II showed higher level and the protection was observed in the dose dependent mannar as with the highest dose

Total Haemoglobin was observed as GI (13.06 ±0.19), GII (7.04 ± 2.14), GIII (14.34 ± 2.00), GIV (11.11±1.00), GV (12.18 ± 1.10), GVI (13.06 ± 0.56). Group II showed lower level of Hb while increase level are shown in dose dependent manner with respect to untreated group II .The methanolic extract of *Nitophyllum marginale* show potential with similar values observed with standard groupIII. In alloxan-induced diabetic rats, excess blood glucose reacts with hemoglobin, reducing total hemoglobin levels(Marudamuthu, 2015). Administration of *Nitophyllum marginale* prevents elevated glycosylated hemoglobin, potentially through improved glycemic control. Alloxan induces diabetes by damaging pancreatic beta cells, leading to insulin deficiency, elevated blood glucose, and subsequent glycation of hemoglobin, contributing to decreased total hemoglobin levels.

Diabetes mellitus commonly results in an elevated serum lipid profile, which heightens the risk of cardiovascular disease. This elevation in serum lipids is associated with the uncontrolled action of lipolytic hormones on fat deposits, primarily due to diminished insulin activity (Shah & Khan, 2014). Under normal conditions, insulin activates the enzyme lipoprotein lipase, which hydrolyzes triglycerides. In diabetic circumstances, the absence of insulin inhibits the activation of lipoprotein lipase, resulting to hypertriglyceridemia (Albai et al., 2017). Furthermore, insulin deficiency is linked to hypercholesterolemia because insulin inhibits HMG-CoA reductase, a key enzyme involved in the metabolism of cholesterol-rich LDL particles. The pathways responsible for growing hypertriglyceridemia and hypercholesterolemia in uncontrolled diabetes involve numerous metabolic problems that

emerge progressively(Athyros et al., 2018). In our investigation, diabetic rats had hypercholesterolemia and hypertriglyceridemia, and treatment with Nitophyllum marginale extract significantly lowered both cholesterol and triglyceride levels. This suggests that *Nitophyllum marginal*e extract treatment can counteract or be beneficial in alleviating lipid profile complications.

5.8.2.5 Effect of treatment on Antioxidant enzymes

Table 27: Effect of *N.marginale* extract on antioxidants enzyme

Parameters (µmol $min^{-1}mg^{-1}prot$)	**Group-I**	**Group-II**	**Group-III**	**Group-IV**	**Group-V**	**Group-VI**
SOD (liver)	5.21±0.31	1.89±0.21	4.12±0.13 ^{c}	2.08±0.06 ^{c}	2.67±0.03 ^{c}	3.79±0.04 ^{c}
SOD (Kidney)	8.38±0.68	1.43±0.18	7.62±0.11 ^{c}	3.12±0.19 ^{c}	4.66±0.02 ^{c}	6.88±0.03 ^{c}
CAT (liver)	15.06±0.64	2.65±0.67	13.19±0.89 ^{c}	6.06±0.01 ^{c}	8.26±0.03 ^{c}	11.17±0.04 ^{c}
CAT (Kidney)	15.86±0.62	8.12±0.98	11.24±0.78 ^{c}	8.1±0.04 ^{c}	9.21±0.06 ^{c}	10.07±0.06 ^{c}
GSH(Liver)	8.09±0.24	4.12±0.34	7.38±0.23 ^{c}	6.12±0.18 ^{c}	6.62±0.12 ^{c}	7.02±0.19 ^{c}
GSH(Kidney)	16.13±0.37	10.17±0.21	14.27±0.17 ^{c}	12.42±0.11 ^{c}	12.87±0.1 ^{c}	13.56±0.28 ^{c}
MDA(Liver)	22.02±0.24	36.12±0.31	20.38±0.23 ^{c}	30.12±0.18 ^{c}	27.12±0.12 ^{c}	23.23±0.11 ^{c}
MDA (Kidney)	26.19±0.37	32.17±0.26	24.27±0.17 ^{c}	28.42±0.11 ^{c}	26.03±0.1 ^{c}	23.13±0.13 ^{c}

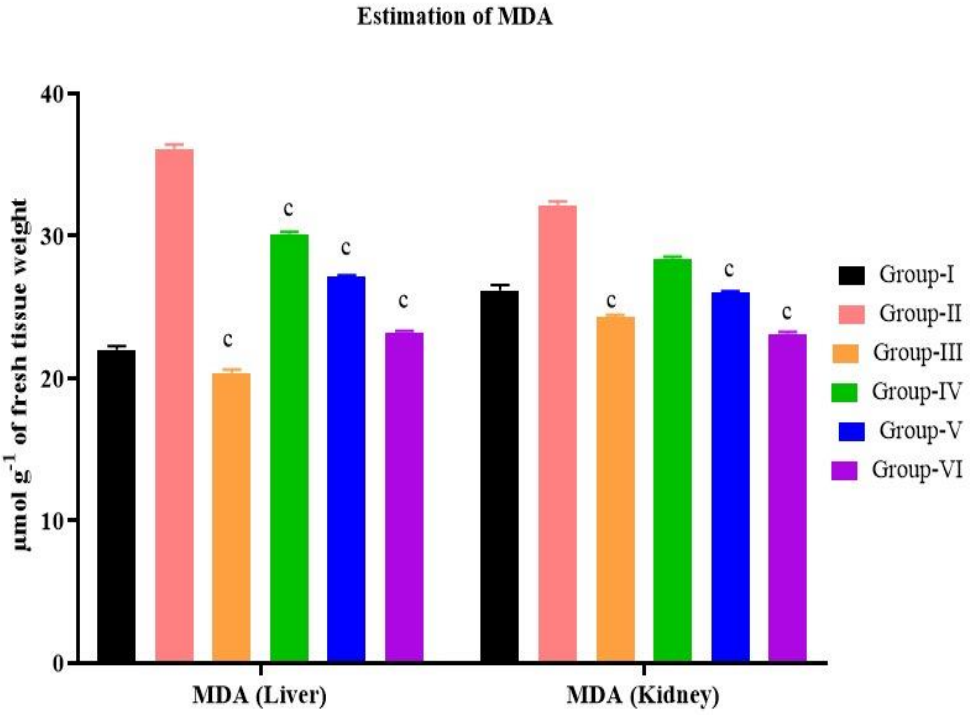


Fig 29: Effects of treatment on antioxidant enzyme (MDA), Group I: normal control (non-diabetic), receiving normal saline, Group II: untreated diabetic control, receiving 150 mg/Kg alloxan monohydrate, Group III: diabetic rats treated by the standard diabetic drug glibenclamide (10 mg/kg), Group IV, V and VI: diabetic rats treated by methanolic extract of *N. marginale* at low, medium and high doses, respectivelyValues represent the Mean± SEM of six rats in each group (n=6). Statistical analysis was performed using one-way analysis of variance with post-hoc testing. c=*p<0.05 compared to the diabetic control.

The antioxidant enzymes were estimated in rats of group received test, standard, normal as well as in alloxan induced groups. The level of CAT was measured in Liver GI (15.06 ± 0.64), GII (2.65 ± 0.67), GIII (13.19±0.89), GIV (6.06 ± 0.01), GV (8.26 ±0.03), GVI (11.17 ±0.04) in group I, II, III, IV, V and VI respectively .The investigation clearly indicated that GII showed lower levels of CAT while protection was observed in dose dependent manner as with the highest dose the protection level as found closer to the normal group of animal even the lower dose also showed protection against elevated levels of CAT. The findings suggest that the methanolic extract shows better results with respect to standard group(Murakami et al., 1995).

The level of CAT was measured in Kidney GI (15.86 ± 0.62), GII (8.12 ± 0.98), GIII (11.24±0.78), GIV (8.1 ± 0.04), GV (9.21 ±0.06), GVI (11.17 ±0.04) in group I, II, III, IV, V and VI respectively

The obtained results reveal that GSH levels in Liver the experimental groups were as follows: GI (8.09±0.24), GII (4.12±0.34), GIII (7.38±0.23), GIV (6.12±0.18), GV (6.62 ±0.12), and GVI (7.02 ±0.19). These values were determined after administering tests, standards, and normal as well as in alloxan-induced groups for Group I, II, III, IV, V, and VI, respectively. The GSH level was measured in Kidney GI (16.13±0.37), GII (10.17±0.21), GIII (14.27±0.17), GIV (12.42±0.11), GV (12.87 ±0.1), and GVI (13.56 ±0.28).The analysis highlights that GII exhibited lower GSH levels, and a dose-dependent protective effect was observed. The highest dose demonstrated a protection level closer to the normal group, while even the lower dose provided protection against elevated GSH levels. These findings suggest that the methanolic extract yielded more favorable results compared to the standard group(Zang et al., 2023).

The results obtained indicate TBARS levels measured in liver the experimental groups: GI (22.02±0.24), GII (36.12±0.31), GIII (20.38±0.23), GIV (30.12±0.18), GV (27.12 ±0.12), and GVI (23.23 ±0.11). These values were determined post-administration of tests, standards, normal, and in alloxan-induced groups for Group I, II, III, IV, V, and VI, respectively. TBARS levels measured in Kidney the experimental groups: GI (26.19±0.37), GII (32.17±0.26), GIII (24.27±0.17), GIV (28.42±0.11), GV (26.03 ±0.1), and GVI (23.13 ±0.13)

Notably, GII exhibited higher TBARS levels, indicating a dose-dependent protective effect. The highest dose exhibited a protection level approaching that of the normal group, and even the lower dose conferred protection against elevated TBARS levels. These results suggest

that the methanolic extract produced more favorable outcomes compared to the standard group, particularly in conjunction with TBARS analysis(Carpena et al., 2022).

SOD level measured in liver was G1(5.21±0.31), GII(1.89±0.21), GIII(4.21±0.13), GIV(2.08±0.06), GV(2.67±0.03) and GVI(3.79±0.04) respectively and the level were measured in Kidney was G1(8.38±0.37), GII (1.43±0.21), GIII(7.62±0.11), G4IV(3.12±0.19), GV(4.66±0.02) and GVI (6.88±0.03).Notably, GII exhibited lower SOD levels, indicating a dose-dependent protective effect. The highest dose exhibited a protection level approaching that of the normal group, and even the lower dose conferred protection against decreased SOD levels. These results suggest that the methanolic extract produced more favorable outcomes compared to the standard group, particularly in conjunction with SOD analysis

Catalase, a pivotal antioxidant enzyme, plays a crucial role in cellular defense mechanisms by efficiently breaking down hydrogen peroxide into water and oxygen, thereby preventing the accumulation of reactive oxygen species (ROS) and mitigating oxidative stress. Its significance is highlighted in populations such as the lesser grain borer, where its differential activity, along with other antioxidants, is associated with resistance to the fumigant phosphine in grain storage (Ranjith et al., 2023). Additionally, catalase exhibits altered expression in tumors, although the underlying molecular regulatory mechanisms remain incompletely understood. Recognized as one of the oldest antioxidant enzymes, catalase's collaboration with other cellular defenses underscores its potential therapeutic relevance in managing conditions associated with oxidative stress, emphasizing the need for further exploration and understanding of its intricate roles in maintaining cellular homeostasis(Glorieux & Calderon, et.al., 2017).

Glutathione (GSH), a pivotal tri-peptide composed of γ-L-glutamyl-L-cysteinyl-glycine, stands at the forefront of cellular redox regulation, detoxification, and iron metabolism. Abundantly synthesized in the cytosol, GSH plays a multifaceted role in safeguarding cellular health. Its functions extend beyond antioxidant defense, encompassing protection against reactive oxygen species (ROS) and nitrogen species, contributing to the maintenance of cellular thiol status. Moreover, GSH's significance is underscored in various physiological processes, particularly in the brain, where it serves as a vital intracellular antioxidant(Dwivedi et al., 2020). As a key component of the AsA–GSH cycle, GSH collaborates with antioxidant enzymes, including superoxide dismutase, catalases, guaiacol

peroxidase, and ascorbate peroxidase, as demonstrated in studies on wheat tolerance to cadmium exposure(Qin et al., 2018). The intricate interplay between GSH and antioxidant systems reflects its central role in cellular homeostasis and resilience against oxidative stress, making it a subject of continued exploration in understanding and promoting overall well-being.

The Thiobarbituric Acid Reactive Substances (TBARS) assay is a widely employed method for assessing lipid peroxidation in biological samples. Lipid peroxidation occurs when free radicals attack lipids, particularly fats in cell membranes, resulting in oxidative damage. TBARS specifically targets the measurement of malonaldehyde (MDA), a secondary product formed during the process of lipid oxidation. The protocol involves a colorimetric method to estimate MDA levels, providing researchers with a quantitative measure of lipid peroxidation (Aguilar Diaz et.al., 2020). This methodology proves valuable in evaluating oxidative stress and lipid damage, offering insights into cellular health, inflammation, and disease prevention. By quantifying MDA levels using TBARS, scientists can monitor oxidative stress levels, crucial for preventing non-communicable diseases (NCDs) (Zeb & Ullah, 2016). The assessment of oxidative stress is pivotal in understanding the impact of free radicals on cellular structures and functions, emphasizing the importance of antioxidants in mitigating oxidative damage and maintaining overall health.

Superoxide dismutase (SOD) is an important endogenous antioxidant enzyme that plays a crucial role in protecting algal cells from oxidative stress. Seaweeds, which are a rich source of various antioxidants, have been found to contain SOD and other peroxidases that protect them from oxidative damage. The presence of these antioxidants in seaweeds makes them a promising source for sustainable development and potential applications in various fields, including food, pharmaceuticals, and cosmetics(Zheng et al., 2023). SOD along with other antioxidants, helps seaweeds to cope with the oxidative stress generated by their exposure to various environmental factors , such as light , temperature and salinity changes as well as pollutants and other stressors(Pan et al., 2006). The ability of seaweeds to adapt to different environmental conditions is partly due to their antioxidant systems, which help maintain the balance between the reactive oxygen species (ROS) and their elimination(Das & Roychoudhury, 2014). Several studies have investigated the antioxidant properties of seaweeds, including their SOD activity, and have found that they vary depending on the species, location and growth conditions (M. M. Ismail et al., 2023). A study on the phenolic compounds and antioxidant activities of selected species of seaweeds from Danish coasts

found that the antioxidant activities of the seaweed were significantly correlated with their phenolic content(El-Beltagi et al., 2022). Another study on the chemical compositions of *Gracilaria dura* and *Hypnea mucisformis* (Rhodophyta) from Corsican lagoons found that these seaweeds contained various antioxidant compounds, including SOD, catalase and glutathione peroxidase (El-Beltagi et al., 2022).The antioxidant properties of seaweeds have attracted considerable interest in recent years due to their potential health benefits for humans and animals. Several studies have reported that seaweed-derived antioxidants , including SOD have various biological activities, such as anti-inflammatory, anticancer and neuroprotective effects.

5.8.2.6 Histopathology of Liver and kidney:

The histological appearance of the liver tissue sections of rats in the control group (GI) revealed normal hepatic architecture (Fig.30). Experimental group (GII) Section from liver showed sinusoidal congestion as well as central and portal vein also showed congestion. Vacuolated granular cytoplasm was observed. Hepatocytes were swollen. These alterations were normalised in rats treated with methanolic extracts of *N. marginale* (Groups IV, V, and VI in Fig. 30), while as medium and high doses of methanolic extracts of *N. marginale* provided better inflammation prevention.

Histological examination of sections from pancreas of GI shows Normal acinar parenchyma of pancreas along with ducts and islets of Langerhans. (Fig. 30). Experimental group (GII)' Pancreatic tissue show swollen and vacuolated acinar cells along with depletion of islets of Langerhans (Fig 30). Sections show normal exocrine and endocrine components. Acinar cells was found normal and regeneration of islets of Langerhans was observed (G IV, V, and VI) with methanolic extracts of *N. marginale* . The group treated with methanolic extracts of *N. marginale* exhibited healing characteristics. More protection was reported in medium and high doses of *N. marginale* methanolic extracts

Histological examination of sections from both the kidneys of GI shows normal architecture. (Fig. 30). Experimental group (GII)' kidneys showed Sections from both the kidneys show severe haemorrhages between intertubular spaces, degeneration epithelial linings of tubules, inflammatory cells, in left kidney nuclear clouding and thickened basement membranes were observed (Fig 30). All necrotic alterations were absent in treatment groups (G IV, V, and VI) with methanolic extracts of *N. marginale* . The group treated with methanolic extracts of *N. marginale* exhibited healing characteristics such as normal glomerulus, absence of

inflammatory cells, normal basement membrane, and capillaries. More protection was reported in medium and high doses of *N. marginale* methanolic extracts.

The liver is responsible for insulin clearance and inflammatory cytokine production, which helps in maintaining normal glucose levels during fasting and postprandial states. Serum enzymes like ALT, AST, and ALP can indicate hepatic injury, as they leak from the cytosol into the bloodstream due to tissue destruction(WEST & ZIMMERMAN, 1959). 36 Diabetic rats exhibited considerably higher levels of ALT, AST, and ALP. After 21 days of therapy with methanolic extract of *N.marginale* at medium and high dose mg/kg b.w., there was a considerable decrease compared to the first day. Treatment with *N.marginale* significantly decreased ALT, AST, and ALP activity compared to diabetic control rats($p<0.05$)(Anandakirouchenane et al., 2013). A comparative discussion on the histopathological effects of seaweed extracts on the liver, kidney and pancreas in alloxan-induced diabetic models highlights the therapeutic potential of these marine organisms in mitigating diabetes related organ damage. In a study involving *Turbinaria decurrens*, rats treated with acetone extract at doses of 150mg/kg and 300mg/kg exhibited significant improvements in histopathological parameters of the liver and pancreas compared to untreated diabetic controls. The treated rats showed reduced hepatic necrosis and inflammation, with liver architecture appearing more normal, while the pancreas displayed less edema and preserved islet integrity, indicating a protective effect against alloxan-induced damage. Similarly, research on *Sargassum longiotome* demonstrated that treatment significantly reduced liver enzymes (SGOT, SGPT) and improved histological features in both liver and kidneys. The treated groups showed less congestion and fibrosis in the liver, while kidney sections revealed reduced glomerular damage and tubular degeneration compared to untreated diabetic rats. Furthermore, *ulva lactuca* extracts were associated with decreased oxidative stress markers and improved overall tissue morphology in the liver and kidneys, supporting their role in protecting against diabetes-induced organ damage. These findings collectively suggest that seaweed extracts can effectively ameliorate histopathological alteration in vital organs caused by diabetes, highlighting their potential as natural therapeutic agents in managing diabetes and its complications.

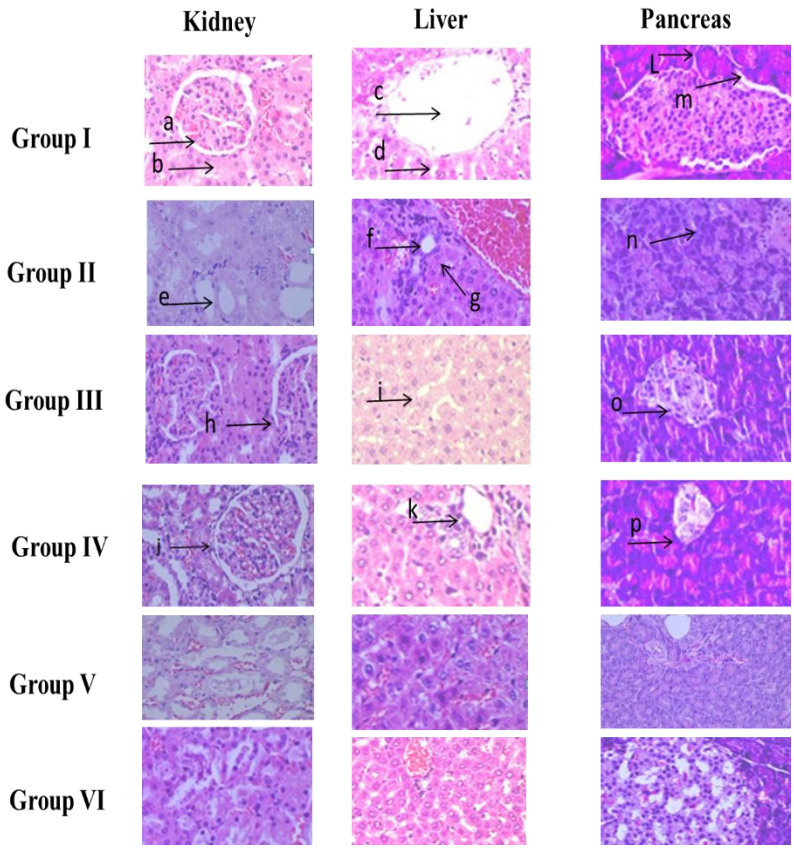


Fig 30: Histopathology of liver, kidney and pancreas photomicrograph of GI (Control), GII (Diabetic control), GIII (glibenclamide), GIV (*N.marginale* extract treated (200mg/kgb.w), GV (400mg/kgb.w), and GVI (800mg/kgb.w)

(a) , **(b)** represents tissue sections from the kidney shows normal architecture, **(c) ,(d)** represents sections from liver show unremarkable histology, **(e)** represents degenerated epithelial linings of tubules inflammatory cells in kidney , **(f), (g)** represents central vein and degenerated hepatic cells, **(h)** represents sections from the kidneys show significant improvement, **(i)** represents unremarkable histology, **(j)** represents mild and improvement in inflammatory cells and basement membrane in kidney with low dose *N.marginale* extract, and rest also improvement seen in medium(400mg/kg b.w) and high dose (800mg/kg b.w) **(k)** represents sections from liver show unremarkable histology with treated low dose (200mg/kg b.w) and also seen improvement in medium and high dose with treated *N.marginale* extract**.(l),(m)** represents normal tubules and islets of Langerhans, **(n)** represents degeneration and depletion of islets of Langerhans,**(o)** Normal acinar parenchyma of pancreas along with ducts and islets of Langerhans, **(p)** Mild degenerative changes in both exocrine and endocrine components at low dose and rest of improvement seen in medium and high dose of *N.marginale* extract.

CHAPTER-6

SUMMARY

Summary

This study delves into Type 2 diabetes mellitus (T2DM), a multifaceted metabolous state considerable by glucose intolerance, imperfect pancreatic β-cell function, and disturbances in glucose regulation. The increasing prevalence of T2DM worldwide underscores the urgent need for novel and holistic treatment strategies. In recent years, natural products have garnered significant interest owing to their various bioactive compounds, presenting opportunities for pharmaceutical and nutraceutical advancement. Within the realm of natural sources, seaweeds have surfaced as a rich repository of bioactive molecules with a wide array of pharmacological benefits.

Nitophyllum marginale, a marine algae species, was sourced from the Gulf of Mannar's coastal region, specifically Mandapam in Tamil Nadu, India, renowned for its biodiversity. Its presence underscores its adaptation to unique environmental conditions and highlights its ecological importance. Studying *N.marginale* from this area can yield insights into its distribution, seasonal variations, and ecological role in the Gulf of Mannar's marine ecosystem.

Bioactive compounds from *N.marginale* were extracted using the Soxhlet method with chloroform and methanol solvents. The dried seaweed powder was placed in a thimble, and the solvent was continuously circulated through the sample for extraction. This controlled process yielded a diverse range of phytochemicals and antioxidants, offering insights into *N.marginale* chemical composition and potential health benefits.

Phytochemical analysis of seaweeds extracts, such as those from *N. marginale*, involves both qualitative and quantitative assessments of various bioactive compounds. In this study, the existence of alkaloids, flavonoids, nitrogen compounds, carbohydrates, and total phenolic content was investigated as preliminary phytochemical contents. By conducting both qualitative and quantitative analyses of these phytochemicals in *N. marginale*, can gain insights into the chemical composition and promising well-being assets of the algae extract, pavement the approach for further pharmacological and nutraceutical applications.

In vitro studies assessing the antioxidant activity of compounds, such as those found in *N. marginale*, often utilize various assays to evaluate their effectiveness in scavenging free radicals and reducing oxidative stress. Common assays include DPPH (1, 1-diphenyl-2-picrylhydrazyl), ABTS (2, 2’-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)), NO (nitric

oxide), LPO (lipid peroxidation), as well as H_2O_2 (hydrogen peroxide) Using methanol and chloroform seaweed extract. Methanol extract yield maximum inhibition with respect to chloroform extract of *N.Marginale*. These assay support potential of compounds from *N.marginale* to combat oxidative stress, protect against cellular damage, and potentially offer therapeutic benefits in conditions associated with oxidative imbalance.

α-Amylase and α-glucosidase inhibitory assay were also conducted using both extract but Methanol extract exhibited superior inhibition with respect to the chloroform extract of *N. marginale*.

The study on cytotoxicity effects of chloroform and methanol extracts of *N.marginale* on 3T3 cell lines showed that the test samples exhibited high cytotoxicity at higher concentrations after 48 hours of treatment. Specifically, the IC50 concentrations for the chloroform as well as methanol extracts was measured to be 192.80 μg/ml and 185.42 μg/ml, against the 3T3 cell line. This suggests that increasing the concentration of the test samples results in higher cytotoxicity against 3T3 cell lines. This findings could be convenient in appreciation the potential toxicity of extracts and in determining the appropriate concentrations for further studies.

The plan for acute toxicity study as per OECD 423 and invivo study was approved by, Animal experimentation ethics committee (IAEC) of Lovely Institute of Technology (Pharmacy), Lovely Professional university (LPU), Punjab, India, under the protocol number LPU/IAEC/2023/34.

The acute oral toxicity study of the methanolic extract of *N. marginale* were performed using OECD -423. The extract was administered as a single dose at levels of 2000, 300, and 50mg/kg body weight. The results showed methanolic obtained of *N. marginale* could not generate indications of toxicity or death even at the maximum amount of 2000 mg/kg Body mass. Therefore, lack in toxicity and mortality at the highest dose tested suggests that the methanolic extract of *N. marginale* has a wide margin of safety for acute oral exposure in rats. The acute oral toxicity of *Nitophyllum marginale* has been approved that is not poisonous to animals and can therefore be employed in research.

The anti-diabetic study of *Nitophyllum marginale* in Alloxan-induced Wistar rats showed that the high dose (800 mg/kg) of the extract significantly reduced blood glucose levels over 21 days compared to the low dose and diabetic control groups.

The treatment's effect on liver function was evaluated across six groups (normal, diabetic, standard, and extract-treated), with SGOT, SGPT, and ALP levels recorded for each, showing that the extract-treated groups had enzyme levels closer to normal than the diabetic group. The effect of treatment on lipid profile was estimated in all 6 groups including normal, diabetic, standard and extract treated groups, the values were measured in 21st day.

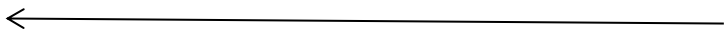
The triglycerides (TG), HDL, VLDL, LDL, and total cholesterol (TC) levels were measured across six groups (normal, diabetic, standard, and extract-treated). The results showed that TG levels were highest in the diabetic group, while the extract-treated groups exhibited TG levels closer to normal. HDL levels were highest in the normal group, with extract-treated groups showing moderately improved levels compared to the diabetic group. VLDL, LDL, and TC levels were notably elevated in the diabetic group but were closer to normal in the extract-treated groups, indicating a potential lipid-regulating effect of the treatment.

The levels of antioxidant enzymes, including Superoxide Dismutase (SOD), Catalase (CAT), Glutathione (GSH), and Malondialdehyde (MDA as TBARS), were measured in both liver and kidney tissues across six groups (normal, diabetic, standard, and extract-treated). SOD levels in the liver and kidney were highest in the normal group, with moderate improvement in extract-treated groups compared to the diabetic group. CAT levels in both tissues were also elevated in the normal group, with notable increases in the extract-treated groups. GSH levels were highest in the normal group for both liver and kidney, with extract-treated groups showing levels closer to normal than diabetic controls. MDA levels, indicating lipid peroxidation, were highest in the diabetic group for both tissues but were closer to normal levels in the extract-treated groups, suggesting an antioxidant effect of the treatment.

The results of invivo study clearly revealed indicated that extract of *Nitophyllum marginale* showed pronounced amelioration in diabetic condition in rats. All the doses of *Nitophyllum marginale* showed dose dependent response in the treatment of alloxan induced diabetic condition. The reason for these effects may be due to its phytoconstituents present in it i.e methanolic extract. This treatment was also evident with antioxidant effect *Nitophyllum marginale* . Finally its antidiabetic effect was supported by histopathological responses where

regeneration was observed in all the groups treated with *Nitophyllum marginale* at their low, medium and high doses. Consequently the response is as follows:

Standard > Nitophyllum marginale (High dose) > (Medium dose) > *Nitophyllum marginale* (Low dose)



Increase in anti-diabetic effect

Chapter-7

CONCLUSION

Conclusion

This Investigation suggests that the preliminary phytoconstituents are able to protect the oxidative stress even they can neutralise the reactive oxygen species. Our finding suggests that methanolic extracts are superior over chloroform extracts in all aspects of evaluation. *Nitophyllum marginale* methanolic extract can be also categorized as the drug of class 5 according to globally hormonised classification system. The present study also demonstrated that medum and high doses of methanolic extract can reduce the alloxan induced diabetes as well as its associated problems (SGOT, SGPT), TGL, HDL, LDL, VLDL etc. Hence it can be a new attractive for the treatment of diabetes.

Future prospect:

The study need full mechanistic approach to find out for the treatment of diabetes even the study also need if any formulation development can be selected for quick relief of diabetes.

BIBLIOGRAPHY

References

A D Baron 1, M Laakso, G Brechtel, S. V. E. (n.d.). Reduced capacity and affinity of skeletal muscle for insulin-mediated glucose uptake in noninsulin-dependent diabetic subjects. Effects of insulin therapy. *J Clin Invest . 1991 Apr;87(4):1186-94*. https://doi.org/10.1172/JCI115117

ABA, P. E. (2016). Glycaemic, Lipidaemic and Antioxidant Profiles of Alloxan-Induced Diabetic Wistar Rats Treated with Glibenclamide and Aqueous Extract of *Gongronema latifolium* (Benth). *Notulae Scientia Biologicae*, *8*(4), 408–413. https://doi.org/10.15835/nsb849857

Aba, P. E., & Edeh, M. N. (2019). Age Susceptibility of Wistar Rats to Alloxan-Induced Diabetes: A Paradox. *Notulae Scientia Biologicae*, *11*(2), 191–195. https://doi.org/10.15835/nsb11210438

Abbas, F., Ke, Y., Yu, R., Yue, Y., Amanullah, S., Jahangir, M. M., & Fan, Y. (2017). Volatile terpenoids: multiple functions, biosynthesis, modulation and manipulation by genetic engineering. *Planta*, *246*(5), 803–816. https://doi.org/10.1007/s00425-017-2749-x

Abrams, D., Metcalf, D., & Hojjatie, M. (2014). Determination of Kjeldahl nitrogen in fertilizers by AOAC official methodSM 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

Adomi, P., Owhe-Ureghe, U., & Asagba, S. (2017). Evaluation of the Toxicity of Phyllanthus Amarus in Wistar Albino Rats. *African Journal of Cellular Pathology*, *8*(5), 27–35. https://doi.org/10.5897/ajcpath17.005

Agardh, J. (2022). *ANALYSIS OF PHYTOCHEMICAL , BIOCHEMICAL PROFILE AND ANTIOXIDANT ACTIVITY OF Sargassum*. *10*(4), 870–879.

Aguilar Diaz De Leon, J., & Borges, C. R. (2020). Evaluation of oxidative stress in biological samples using the thiobarbituric acid reactive substances assay. *Journal of Visualized Experiments*, *2020*(159), 1–10. https://doi.org/10.3791/61122

Aina, O., Bakare, O. O., Daniel, A. I., Gokul, A., Beukes, D. R., Fadaka, A. O., Keyster, M., & Klein, A. (2022). Seaweed-Derived Phenolic Compounds in Growth Promotion and Stress Alleviation in Plants. *Life*, *12*(10). https://doi.org/10.3390/life12101548

Akhter, M., Ullah, M. R., Khan, A. B. S., Hasan, M. M., Yasmin, F., Bosu, A., Haque, M. A., Islam, M. M., Islam, M. A., & Mahmud, Y. (2024). Assessment of different solvents effect on bioactive compounds, antioxidant activity and nutritional formation of red seaweed, Gracilariopsis longissima, from Bay of Bengal, Bangladesh. *Discover Food*, *4*(1). https://doi.org/10.1007/s44187-023-00069-1

Al-Afifi, N. A., Alabsi, A. M., Bakri, M. M., & Ramanathan, A. (2018). Acute and sub-acute oral toxicity of Dracaena cinnabari resin methanol extract in rats. *BMC Complementary and Alternative Medicine*, *18*(1), 1–14. https://doi.org/10.1186/s12906-018-2110-3

Albai, O., Roman, D., & Frandes, M. (2017). *Therapeutics and Clinical Risk Management independent risk factor for acute pancreatitis in patients with type 2 diabetes mellitus*

Hypertriglyceridemia , an important and independent risk factor for acute pancreatitis in patients with type 2 diabetes mell. https://doi.org/10.2147/TCRM.S134560

Alberti, K. G. M. M., & Zimmet, P. Z. (1998). Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: Diagnosis and classification of diabetes mellitus. Provisional report of a WHO consultation. *Diabetic Medicine*, *15*(7), 539–553. https://doi.org/10.1002/(SICI)1096-9136(199807)15:7<539::AID-DIA668>3.0.CO;2-S

Ali, O., & Ramsubhag, A. (2021). *Biostimulant Properties of Seaweed Extracts in Plants : Implications towards Sustainable Crop Production*.

Alshehri, M. A., Aziz, A. T., Alzahrani, O., Alasmari, A., Ibrahim, S., Osman, G., & Bahattab, O. (2019). DNA-barcoding and Species Identification for some Saudi Arabia Seaweeds using rbcL Gene. *Journal of Pure and Applied Microbiology*, *13*(4), 2035–2044. https://doi.org/10.22207/JPAM.13.4.15

Amaro, H. M., Pagels, F., Tavares, T. G., Costa, I., Sousa-Pinto, I., & Guedes, A. C. (2022). Antioxidant and Anti-Inflammatory Potential of Seaweed Extracts as Functional Ingredients. *Hydrobiology*, *1*(4), 469–482. https://doi.org/10.3390/hydrobiology1040028

Anaduaka, E. G., Ogugua, V. N., Agu, C. V, & Okonkwo, C. C. (2014). Ethanol extracts of Newbouldia laevis stem and leaves modulate serum liver marker enzymes and antioxidant enzymes activities in diabetic rats. *African Journal of Biotechnology*, *13*(22), 2265–2272. https://doi.org/10.5897/ajb2014.13753

Anandakirouchenane, E., Sarath Chandiran, I., Kanimozhi, V., & Kadalmani, B. (2013). Antioxidant and protective effect of Curculigo orchioides on liver, pancreas and kidney tissue inalloxan induced diabetic experimental rats. *Drug Invention Today*, *5*(3), 192–200. https://doi.org/10.1016/j.dit.2013.08.002

Angell, A. R., Mata, L., de Nys, R., & Paul, N. A. (2016). The protein content of seaweeds: a universal nitrogen-to-protein conversion factor of five. *Journal of Applied Phycology*, *28*(1), 511–524. https://doi.org/10.1007/s10811-015-0650-1

Antioxidants, E. O. F. (2017). *Original Research Paper Botany Dr . Chitra . G Razeena P N.* *10*, 9–11.

Antioxidants, E. O. F., & Of, P. S. (2017). *Original Research Paper Botany Dr . Chitra . G Razeena P N*. *10*, 9–11.

Ar, S. R. K. U. M., Vya, B. D. I., & Alarko, A. N. D. C. M. (2017). *Antidia betic a ctivity of novel a lga l formula tions (Pa dina tetra stroma tica a nd Turbina ria conoides) a ga inst Streptozotocin induced dia betes in a lbino ra ts*. *9*(2), 54–58.

Arive, P. L. C., Inquimboy, I. H., & No, N. L. I. (2017). *In Vitro Antioxidant Activity of Selected Seaweeds in the Philippines*. *9*(2), 212–216. https://doi.org/10.13140/RG.2.2.11265.30567

Arora, S., & Suvartan, R. (2020). PHYTOCHEMICALS : BENEFITS , CONCERNS AND CHALLENGES Advancement in Advancement in Functional Food Ingredients Advancement in Functional Food Ingredients Functional Food Ingredients Book on Advancement in Functional Food Ingredients :... *Advancement in Functional Food Ingredient*, *July*, 205–227.

Arulkumar, A., Rosemary, T., Paramasivam, S., & Rajendran, R. B. (2018). Phytochemical composition, in vitro antioxidant, antibacterial potential and GC-MS analysis of red seaweeds (Gracilaria corticata and Gracilaria edulis) from Palk Bay, India. *Biocatalysis and Agricultural Biotechnology*, *15*, 63–71. https://doi.org/10.1016/j.bcab.2018.05.008

Arunkumar, K., Raja, R., Kumar, V. B. S., Joseph, A., Shilpa, T., & Carvalho, I. S. (2021). Antioxidant and cytotoxic activities of sulfated polysaccharides from five different edible seaweeds. *Journal of Food Measurement and Characterization*, *15*(1), 567–576. https://doi.org/10.1007/s11694-020-00661-4

Athyros, V. G., Doumas, M., Imprialos, K. P., Stavropoulos, K., Georgianou, E., Katsimardou, A., & Karagiannis, A. (2018). *Diabetes and lipid metabolism*. 61–67.

Avinash Kumar Jha, N. S. (n.d.). Extraction of bioactive compounds from plant materials using combination of various novel methods: A review. *Trends in Food Science & Technology*, *119*(579–591).

Awah, F. M., & Verla, A. W. (2010). Antioxidant activity, nitric oxide scavenging activity and phenolic contents of Ocimum gratissimum leaf extract. *Journal of Medicinal Plants Research*, *4*(23), 2479–2487. https://doi.org/10.5897/jmpr10.262

Baba, W. N., Tabasum, Q., Muzzaffar, S., Masoodi, F. A., Wani, I., Ganie, S. A., & Bhat, M. M. (2018). Author ' s Accepted Manuscript. *Food Bioscience*. https://doi.org/10.1016/j.fbio.2018.02.009

Bailey, C. J., & Day, C. (2003). Antidiabetic drugs CO RE (C PY PR AR RI CT LOG M IO Y ED N) L IN. *The British Journal of Cardiology*, *10*(2), 128–136.

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*, *27*(4). https://doi.org/10.3390/molecules27041326

Basir, A., Desniar, Ristyanti, W. K., & Tarman, K. (2020). Physical treatments to induce the antibacterial and antioxidant activities of green algae Halimeda sp. from Seribu Islands, North Jakarta, Indonesia. *IOP Conference Series: Earth and Environmental Science*, *414*(1), 0–6. https://doi.org/10.1088/1755-1315/414/1/012002

Baynest, H. W. (2015). Classification, Pathophysiology, Diagnosis and Management of Diabetes Mellitus. *Journal of Diabetes & Metabolism*, *06*(05). https://doi.org/10.4172/2155-6156.1000541

Bayro, A. M., Manlusoc, J. K., Alonte, R., Caniel, C., Conde, P., & Embralino, C. (2021). Preliminary Characterization, Antioxidant and Antiproliferative Properties of Polysaccharide from Caulerpa taxifolia. *Pharmaceutical Sciences and Research*, *8*(1), 30–36. https://doi.org/10.7454/psr.v8i1.1102

Benita, M., Dubinsky, Z., & Iluz, D. (2018). <i>Padina pavonica</i>: Morphology and Calcification Functions and Mechanism. *American Journal of Plant Sciences*, *09*(06), 1156–1168. https://doi.org/10.4236/ajps.2018.96087

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

Bito, T., & Watanabe, F. (2022). Seaweeds as a source of vitamin B12. *Sustainable Global Resources of Seaweeds Volume 2: Food, Pharmaceutical and Health Applications*, 339–350. https://doi.org/10.1007/978-3-030-92174-3_18

Box, A., Sureda, A., Terrados, J., Pons, A., & Deudero, S. (2008). Antioxidant response and caulerpenyne production of the alien Caulerpa taxifolia (Vahl) epiphytized by the invasive algae Lophocladia lallemandii (Montagne). *Journal of Experimental Marine Biology and Ecology*, *364*(1), 24–28. https://doi.org/10.1016/j.jembe.2008.06.029

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), 156–161. https://doi.org/10.1080/13880200601113131

Čagalj, M., Skroza, D., Tabanelli, G., Özogul, F., & Šimat, V. (2021). Maximizing the antioxidant capacity of Padina pavonica by Choosing the Right drying and extraction methods. *Processes*, *9*(4), 1–15. https://doi.org/10.3390/pr9040587

Cano, A., Maestre, A. B., Hernández-Ruiz, J., & Arnao, M. B. (2023). ABTS/TAC Methodology: Main Milestones and Recent Applications. *Processes*, *11*(1). https://doi.org/10.3390/pr11010185

Carpena, M., Chamorro, F., Otero, P., Lourenc, C., Cao, H., & Prieto, M. A. (2022). *Biological properties and potential of compounds extracted from red seaweeds* (Vol. 0123456789). https://doi.org/10.1007/s11101-022-09826-z

Chac, L. D., & Thinh, B. B. (2023). Species Identification through DNA Barcoding and Its Applications: A Review. *Biology Bulletin*, *50*(6), 1143–1156. https://doi.org/10.1134/S106235902360229X

Chakraborty, K., Joseph, D., & Praveen, N. K. (2015). Antioxidant activities and phenolic contents of three red seaweeds (Division: Rhodophyta) harvested from the Gulf of Mannar of Peninsular India. *Journal of Food Science and Technology*, *52*(4), 1924–1935. https://doi.org/10.1007/s13197-013-1189-2

Chaudhury, A., Duvoor, C., Reddy Dendi, V. S., Kraleti, S., Chada, A., Ravilla, R., Marco, A., Shekhawat, N. S., Montales, M. T., Kuriakose, K., Sasapu, A., Beebe, A., Patil, N., Musham, C. K., Lohani, G. P., & Mirza, W. (2017). Clinical Review of Antidiabetic Drugs: Implications for Type 2 Diabetes Mellitus Management. *Frontiers in Endocrinology*, *8*(January). https://doi.org/10.3389/fendo.2017.00006

Chaves, N., Santiago, A., & Alías, J. C. (2020). Quantification of the antioxidant activity of plant extracts: Analysis of sensitivity and hierarchization based on the method used. *Antioxidants*, *9*(1). https://doi.org/10.3390/antiox9010076

Chawla, R. (2013). Chapter-05 Classification of Diabetes. *Essentials of Blood Banking*, 36–40. https://doi.org/10.5005/jp/books/12154_5

Chen, B., Li, Y., Li, W., Ye, S., Zhu, L., & Ding, Y. (2023). Antitumor Activity and Mechanism of Terpenoids in Seaweeds Based on Literature Review and Network Pharmacology. *Advanced Biology*. https://doi.org/10.1002/adbi.202300541

Cheng, A. X., Lou, Y. G., Mao, Y. B., Lu, S., Wang, L. J., & Chen, X. Y. (2007). Plant terpenoids: Biosynthesis and ecological functions. *Journal of Integrative Plant Biology*, *49*(2), 179–186. https://doi.org/10.1111/j.1744-7909.2007.00395.x

Cheng, Y.-L., Lee, C.-Y., Huang, Y.-L., Buckner, C. A., Lafrenie, R. M., Dénommée, J. A., Caswell, J. M., Want, D. A., Gan, G. G., Leong, Y. C., Bee, P. C., Chin, E., Teh, A. K. H., Picco, S., Villegas, L., Tonelli, F., Merlo, M., Rigau, J., Diaz, D., … Mathijssen, R. H. J. (2016). We are IntechOpen , the world ' s leading publisher of Open Access books Built by scientists , for scientists TOP 1 %. *Intech*, *11*(tourism), 13. https://www.intechopen.com/books/advanced-biometric-technologies/liveness-detection-in-biometrics

Cherry, P., Yadav, S., Strain, C. R., Allsopp, P. J., Mcsorley, E. M., Ross, R. P., & Stanton, C. (2019). Prebiotics from seaweeds: An ocean of opportunity? *Marine Drugs*, *17*(6), 1–35. https://doi.org/10.3390/md17060327

Cho, C. H., Lu, Y. A., Kim, M. Y., Jeon, Y. J., & Lee, S. H. (2022). Therapeutic Potential of Seaweed-Derived Bioactive Compounds for Cardiovascular Disease Treatment. *Applied Sciences (Switzerland)*, *12*(3). https://doi.org/10.3390/app12031025

Choudhary, B., Chauhan, O. P., & Mishra, A. (2021). Edible Seaweeds: A Potential Novel Source of Bioactive Metabolites and Nutraceuticals With Human Health Benefits. *Frontiers in Marine Science*, *8*(October). https://doi.org/10.3389/fmars.2021.740054

Cojandaraj, L., U, S. P., & Shyamala, M. E. (2020). *ANTIOXIDANT ACTIVITY OF MARINE RED ALGAE – PORTIERIA HORNEMANNII*. *20*, 1075–1081.

Cotas, J., Gomes, L., Pacheco, D., & Pereira, L. (2023). Ecosystem Services Provided by Seaweeds. *Hydrobiology*, *2*(1), 75–96. https://doi.org/10.3390/hydrobiology2010006

CZI. (2012). *Coastal Zones of India*. *April*, 1–609. envfor.nic.in/sites/default/files/Coastal_Zones_of_India.pdf

D. Vijayraja, K. J. (n.d.). *Preliminary phytochemical analysis, in vitro antioxidant and anti-inflammatory activity of Turbinaria ornata (Turner) J. Agardh*. https://doi.org/: 10.5958/0974-360X.2017.00397.3

Das, K., & Roychoudhury, A. (2014). Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. *Frontiers in Environmental Science*, *2*(DEC), 1–13. https://doi.org/10.3389/fenvs.2014.00053

David Leslie, R., Evans-Molina, C., Freund-Brown, J., Buzzetti, R., Dabelea, D., Gillespie, K. M., Goland, R., Jones, A. G., Kacher, M., Phillips, L. S., Rolandsson, O., Wardian, J. L., & Dunne, J. L. (2021). Adult-onset type 1 diabetes: Current understanding and challenges. *Diabetes Care*, *44*(11), 2449–2456. https://doi.org/10.2337/dc21-0770

De Oliveira, L. S., Tschoeke, D. A., De Oliveira, A. S., Hill, L. J., Paradas, W. C., Salgado, L. T., Thompson, C. C., Pereira, R. C., & Thompson, F. L. (2015). New insights on the terpenome of the red seaweed Laurencia dendroidea (florideophyceae, rhodophyta). *Marine Drugs*, *13*(2), 879–902. https://doi.org/10.3390/md13020879

Deepak, P., Josebin, M. P. D., Kasthuridevi, R., Sowmiya, R., Balasubramani, G., Aiswarya, D., & Perumal, P. (2017). GC-MS Metabolite Profiling, Antibacterial, Antidiabetic and

Antioxidant Activities of Brown Seaweeds, Sargassum wightii Greville Ex J. Agardh, 1848 and Stoechospermum marginatum (C. Agardh) Kützing 1843. *Pharmacology, Toxicology and Biomedical Reports*, *3*(2), 27–34. https://doi.org/10.5530/ptb.2017.3.5

Devi, G., Kumari, S. D., & Nadu, T. (2022). *Phytochemical Screening , Gcms Analysis And Biomedical Applications Of Various Extracts From Gracilaria Corticate*. *13*(19113272192001), 6773–6789. https://doi.org/10.47750/pnr.2022.13.S07.820

Devi, K. P., Suganthy, N., Kesika, P., & Pandian, S. K. (2008a). Bioprotective properties of seaweeds: In vitro evaluation of antioxidant activity and antimicrobial activity against food borne bacteria in relation to polyphenolic content. *BMC Complementary and Alternative Medicine*, *8*, 1–11. https://doi.org/10.1186/1472-6882-8-38

Devi, K. P., Suganthy, N., Kesika, P., & Pandian, S. K. (2008b). Bioprotective properties of seaweeds: In vitro evaluation of antioxidant activity and antimicrobial activity against food borne bacteria in relation to polyphenolic content. *BMC Complementary and Alternative Medicine*, *8*(February). https://doi.org/10.1186/1472-6882-8-38

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 1 Mohammed Deyab, 2 Taha Elkatony and 3 Fatma Ward 1 Professor of microbiology, 2 Professor of plant physiology. *International Journal of Engineering Development and Research*, *4*(2), 2321–9939.

Diabetes, D. O. F. (2010). Diagnosis and classification of diabetes mellitus. *Diabetes Care*, *33*(SUPPL. 1). https://doi.org/10.2337/dc10-S062

Dias, V., Bandeira, S., Chaúque, E., Lipassula, M., & Mussagy, A. (2020). Evaluation of Phytocompounds and Chemical Elements Present In Selected Species of Seaweeds, to Sustain Future Quantitative Analysis for Bioactive Compounds. *Journal of Drug Delivery and Therapeutics*, *10*(5), 232–239. https://doi.org/10.22270/jddt.v10i5.4422

DIHARMI, A., EDISON, E., PRIDA, E. A., SUBARYONO, S., & HIDAYAT, T. (2023). Chemical composition, bioactive compounds, antioxidant activity, and inhibitor alpha-glucosidase enzyme of Sargassum sp. *Food Science and Technology*, *43*, 1–7. https://doi.org/10.5327/fst.4623

Dissanayake, I. H., Bandaranayake, U., Keerthirathna, L. R., Manawadu, C., Silva, R. M., Mohamed, B., Rizwan, A., & Peiris, D. C. (2022). Integration of in vitro and in-silico analysis of Caulerpa racemosa against antioxidant, antidiabetic, and anticancer activities. *Scientific Reports*, *12*(1), 1–15. https://doi.org/10.1038/s41598-022-24021-y

Donadee, C., Raat, N. J. H., Kanias, T., Tejero, J., Lee, J. S., Kelley, E. E., Zhao, X., Liu, C., Reynolds, H., Azarov, I., Frizzell, S., Meyer, E. M., Donnenberg, A. D., Qu, L., Triulzi, D., Kim-Shapiro, D. B., & Gladwin, M. T. (2011). Nitric oxide scavenging by red blood cell microparticles and cell-free hemoglobin as a mechanism for the red cell storage lesion. *Circulation*, *124*(4), 465–476. https://doi.org/10.1161/CIRCULATIONAHA.110.008698

Drucker, D. J., & Holst, J. J. (2023). The expanding incretin universe: from basic biology to

clinical translation. *Diabetologia*, *66*(10), 1765–1779. https://doi.org/10.1007/s00125-023-05906-7

Dwivedi, D., Megha, K., Mishra, R., & Mandal, P. K. (2020). Glutathione in Brain: Overview of Its Conformations, Functions, Biochemical Characteristics, Quantitation and Potential Therapeutic Role in Brain Disorders. *Neurochemical Research*, *45*(7), 1461–1480. https://doi.org/10.1007/s11064-020-03030-1

Easwari, S., & Srisudha, S. (2018). *International Journal Of Recent Scientific*. *June*.

El-Beltagi, H. S., Mohamed, A. A., Mohamed, H. I., Ramadan, K. M. A., Barqawi, A. A., & Mansour, A. T. (2022a). Phytochemical and Potential Properties of Seaweeds and Their Recent Applications: A Review. *Marine Drugs*, *20*(6). https://doi.org/10.3390/md20060342

El-Beltagi, H. S., Mohamed, A. A., Mohamed, H. I., Ramadan, K. M. A., Barqawi, A. A., & Mansour, A. T. (2022b). Phytochemical and Potential Properties of Seaweeds and Their Recent Applications: A Review. In *Marine Drugs* (Vol. 20, Issue 6). MDPI. https://doi.org/10.3390/md20060342

El-Katony, T. M., Deyab, M. A., El-Adl, M. F., & Ward, F. M. E.-N. (2021). Extracts of the Brown Alga Dictyota dichotoma (Hudson) J.V. Lamouroux Alleviate Salt Stress in Rice (Oryza sativa L.) During Germination. *Journal of Plant Growth Regulation*, *40*(3), 986–999. https://doi.org/10.1007/s00344-020-10156-7

El Shafay, S., El-Sheekh, M., Bases, E., & El-Shenody, R. (2022). Antioxidant, antidiabetic, anti-inflammatory and anticancer potential of some seaweed extracts. *Food Science and Technology (Brazil)*, *42*. https://doi.org/10.1590/fst.20521

Eloff, J. N. (2019). *Which extractant should be used for the screening of antimicrobials components from plants ? Which extractant should be used for the screening and isolation of antimicrobial components from plants ? 8741*(January). https://doi.org/10.1016/S0378-8741(97)00123-2

Elsie, B. H., & Dhanarajan, M. S. (2010). *Evaluation of antimicrobial activity and phytochemical screening of Gelidium acerosa*. *2*(11), 704–707.

Erlania, & Nyoman Radiarta. (2015). The Use of Seaweeds Aquaculture for Carbon Sequestration: A Strategy for Climate Change Mitigation. *Journal of Geodesy and Geomatics Engineering*, *2*(2). https://doi.org/10.17265/2332-8223/2015.06.006

Fajriah, S., Handayani, S., Sinurat, E., Megawati, M., Darmawan, A., Hariyanti, H., Dewi, R. T., & Septama, A. W. (2020). In vitro Immunomodulatory Effect from Edible Green Seaweed of Caulerpa lentillifera Extracts on Nitric Oxide Production and Phagocytosis Activity of RAW 264.7 Murine Macrophage Cells. *Journal of Young Pharmacists*, *12*(4), 334–337. https://doi.org/10.5530/jyp.2020.12.87

Fayaz, Y., Honarvar, M., & Mooraki, N. (2021). Optimization of Extraction of Antioxidant and Phenolic Compounds of Caulerpa sertularioides by Microwave-Assisted Extraction Process. *Journal of Food Technology and Nutrition*, *18*(Spring 2021), 5–20.

Fazel, S., Hamidreza, M., Rouhollah, G., & Verdian-Rizi, M. (2010). Specimen Copy: Not for sale Spectrophotometric determination of total alkaloids in some Iranian medicinal

plants. *Journal of Applied Horticulture*, *12*(1), 69–70.

Feroz, B. (2018). Saponins from Marine Macroalgae: A Review. *Journal of Marine Science: Research & Development*, *08*(04), 1–8. https://doi.org/10.4172/2155-9910.1000255

Ganesan, M., Eswaran, K., & Mantri, V. A. (2014). *the Seaweed Resources of India*. *December*.

Gani, R., Devi, S., Goundar, S., Reddy, E., Saber, F., Cheng, Y.-L., Lee, C.-Y., Huang, Y.-L., Buckner, C. A., Lafrenie, R. M., Dénommée, J. A., Caswell, J. M., Want, D. A., Gan, G. G., Leong, Y. C., Bee, P. C., Chin, E., Teh, A. K. H., Picco, S., … Mathijssen, R. H. J. (2016). We are IntechOpen , the world ' s leading publisher of Open Access books Built by scientists , for scientists TOP 1 %. *Intech*, *11*(tourism), 13. https://www.intechopen.com/books/advanced-biometric-technologies/liveness-detection-in-biometrics

Gaps, I. (2020). *Seaweed Biodiversity of India : Reviewing Current and Opportunities*.

Gastaldelli, A., Miyazaki, Y., Mahankali, A., Berria, R., Pettiti, M., Buzzigoli, E., Ferrannini, E., & DeFronzo, R. A. (2006). The effect of pioglitazone on the liver: Role of adiponectin. *Diabetes Care*, *29*(10), 2275–2281. https://doi.org/10.2337/dc05-2445

Gazali, M., Jolanda, O., Husni, A., Nurjanah, Majid, F. A. A., Zuriat, & Syafitri, R. (2023). In Vitro α-Amylase and α-Glucosidase Inhibitory Activity of Green Seaweed Halimeda tuna Extract from the Coast of Lhok Bubon, Aceh. *Plants*, *12*(2). https://doi.org/10.3390/plants12020393

Ghorai, N., Ghorai, N., Chakraborty, S., Gucchait, S., Saha, S. K., & Biswas, S. (2012). Estimation of total Terpenoids concentration in plant tissues using a monoterpene, Linalool as standard reagent. *Protocol Exchange*, 1–5. https://doi.org/10.1038/protex.2012.055

Glorieux, C., & Calderon, P. B. (2017). Catalase, a remarkable enzyme: Targeting the oldest antioxidant enzyme to find a new cancer treatment approach. *Biological Chemistry*, *398*(10), 1095–1108. https://doi.org/10.1515/hsz-2017-0131

GOMETI, S. A., OGUGUA, V. N., ODO, C. E., & JOSHUA, P. E. (2014). Effects of some anti-diabetic plants on the hepatic marker enzymes of diabetic rats. *African Journal of Biotechnology*, *13*(7), 905–909. https://doi.org/10.5897/ajb2013.13329

Goossens, G. H., & Blaak, E. E. (2015). Adipose tissue dysfunction and impaired metabolic health in human obesity: A matter of oxygen? *Frontiers in Endocrinology*, *6*(APR), 1–5. https://doi.org/10.3389/fendo.2015.00055

Gopalasatheeskumar, K. (2018). Significant Role of Soxhlet Extraction Process in Phytochemical Research Development of Herbal Formulations for Diabetes View Project. *Mintage Journal of Pharmaceuitcal & Medical Sciences*, *7*(1), 43–47. www.mintagejournals.com43

Gowda, S. G. B., Yifan, C., Gowda, D., Tsuboi, Y., Chiba, H., & Hui, S. P. (2022). Analysis of Antioxidant Lipids in Five Species of Dietary Seaweeds by Liquid Chromatography/Mass Spectrometry. *Antioxidants*, *11*(8), 1–14. https://doi.org/10.3390/antiox11081538

Grace, M. P. (2021). *QUALITATIVE ANALYSIS OF PHYTOCHEMICALS IN SELECTED SEAWEEDS OF MANDAPAM COAST ,. 7838*(May), 144–160.

Graf, E., & Penniston, J. T. (1980). Method for determination of hydrogen peroxide, with its application illustrated by glucose assay. *Clinical Chemistry*, *26*(5), 658–660. https://doi.org/10.1093/clinchem/26.5.0658

Grammatiki, M., Sagar, R., & Ajjan, R. A. (2020). Metformin: Is it Still the First Line in Type 2 Diabetes Management Algorithm? *Current Pharmaceutical Design*, *27*(8), 1061–1067. https://doi.org/10.2174/1381612826666201222154616

Guideline, O., Testing, F. O. R., & Chemicals, O. F. (2001). *Acute Oral Toxicity – Acute Toxic Class Method*. *December*, 1–14.

Gupta, R. N., Pareek, A., Suthar, M., Rathore, G. S., Basniwal, P. K., & Jain, D. (2009). Study of glucose uptake activity of Helicteres isora Linn. fruits in L-6 cell lines. *International Journal of Diabetes in Developing Countries*, *29*(4), 170–173. https://doi.org/10.4103/0973-3930.57349

Harborne;, J. B. (1987). *Metode Fitokimia : Penuntun Cara Modern Menganalisis Tumbuhan*. http://perpustakaan.stifar.ac.id/index.php?p=show_detail&id=448&keywords=harborne

He, L. Y., Li, Y., Niu, S. Q., Bai, J., Liu, S. J., & Guo, J. L. (2023). Polysaccharides from natural resource: ameliorate type 2 diabetes mellitus via regulation of oxidative stress network. *Frontiers in Pharmacology*, *14*(July), 1–20. https://doi.org/10.3389/fphar.2023.1184572

Heinrich, M., Mah, J., & Amirkia, V. (2021). Alkaloids used as medicines: Structural phytochemistry meets biodiversity—An update and forward look. *Molecules*, *26*(7), 1–18. https://doi.org/10.3390/molecules26071836

Hidayati, J. R., Yudiati, E., Pringgenies, D., & Oktaviyanti, D. T. (2020). *Comparative Study on Antioxidant Activities , Total Phenolic Compound and Pigment Contents of Tropical Spirulina platensis , Gracilaria arcuata and Ulva lactuca Extracted in Different Solvents Polarity*. *03012*.

Hossain, M. A., & Pervin, R. (2018). Current Antidiabetic Drugs. In *Nutritional and Therapeutic Interventions for Diabetes and Metabolic Syndrome* (Second Edi). Elsevier Inc. https://doi.org/10.1016/B978-0-12-812019-4.00034-9

Hu, W., Sarengaowa, W., Guan, Y., & Feng, K. (2022). Biosynthesis of Phenolic Compounds and Antioxidant Activity in Fresh-Cut Fruits and Vegetables. *Frontiers in Microbiology*, *13*(May), 1–8. https://doi.org/10.3389/fmicb.2022.906069

Inc ; Jain, N. K., Mishra, P. R., Mishra, M., & Namdeo, A. (1990). 5. Szejtli J. Medicinal applications of Cyclodextrins. *Indian J Pharm Sci*, *4*(December), 353–386.

International Journal of Advances in Pharmaceutics Inclusion of Hydrophilic-Lipophilic Balance (HLB) in the treatment of Psoriasis-A new approach QR Code. (2019). *5*(3), 10–14. https://doi.org/10.7439/ijap

Ismail, G. A., Gheda, S. F., Abo-Shady, A. M., & Abdel-Karim, O. H. (2020). In vitro potential activity of some seaweeds as antioxidants and inhibitors of diabetic enzymes.

Food Science and Technology (Brazil), *40*(3), 681–691. https://doi.org/10.1590/fst.15619

Ismail, M. M., El Zokm, G. M., & Miranda Lopez, J. M. (2023). Nutritional, bioactive compounds content, and antioxidant activity of brown seaweeds from the Red Sea. *Frontiers in Nutrition*, *10*(July). https://doi.org/10.3389/fnut.2023.1210934

Ityodugh, A., Hemen, J., & Elijah, B. (2019). *Evaluatin of Body Weight And Some Morphometric Traits Of Laboratory Albino Rat (Rattus norvegicus)*. *10*(April), 5–8.

Ivanova, N., Gugleva, V., Dobreva, M., Pehlivanov, I., Stefanov, S., & Andonova, V. (2016). We are IntechOpen , the world ' s leading publisher of Open Access books Built by scientists , for scientists TOP 1 %. *Intech*, *i*(tourism), 13.

J Stella Mary 1, P Vinotha, A. M. P. (2012). Screening for in vitro cytotoxic activity of seaweed, Sargassum sp. against Hep-2 and MCF-7 cancer cell lines. *Asian Pac J Cancer Prev . 2012;13(12):6073-6.* https://doi.org/10.7314/apjcp.2012.13.12.6073

Jaber, S. A. (2023). In vitro alpha-amylase and alpha-glucosidase inhibitory activity and in vivo antidiabetic activity of Quercus coccifera (Oak tree) leaves extracts. *Saudi Journal of Biological Sciences*, *30*(7), 103688. https://doi.org/10.1016/j.sjbs.2023.103688

Jagetia, G. C., & Baliga, M. S. (2004). The evaluation of nitric oxide scavenging activity of certain Indian medicinal plants in vitro: A preliminary study. *Journal of Medicinal Food*, *7*(3), 343–348. https://doi.org/10.1089/jmf.2004.7.343

Jaworowska, A., & Murtaza, A. (2023). Seaweed Derived Lipids Are a Potential Anti-Inflammatory Agent: A Review. *International Journal of Environmental Research and Public Health*, *20*(1). https://doi.org/10.3390/ijerph20010730

Jin, H. J., Kim, J. H., Sohn, C. H., DeWreede, R. E., Choi, T. J., Towers, G. H. N., Hudson, J. B., & Hong, Y. K. (1997). Inhibition of Taq DNA polymerase by seaweed extracts from British Columbia, Canada and Korea. In *Journal of Applied Phycology* (Vol. 9, Issue 4, pp. 383–388). https://doi.org/10.1023/A:1007925202219

Jothy, S. L., Zakaria, Z., Chen, Y., Lau, Y. L., Latha, L. Y., & Sasidharan, S. (2011). Acute oral toxicity of methanolic seed extract of Cassia fistula in mice. *Molecules*, *16*(6), 5268–5282. https://doi.org/10.3390/molecules16065268

Joy Lindsey, A. P., Issac, R., Prabha, M. L., Renitta, R. E., Catherine, A., Samrot, A. V., Abirami, S., Prakash, P., & Dhiva, S. (2021). Evaluation of Antidiabetic Activity of Sargassum tenerrimum in Streptozotocin-Induced Diabetic Mice. *Journal of Pure and Applied Microbiology*, *15*(4), 2462–2472. https://doi.org/10.22207/JPAM.15.4.73

K., M., G., T., G. K., D., A., H., & P., A. (2008). Biochemical Composition of Seaweeds from Mandapam Coastal Regions along Southeast Coast of India. *American-Eurasian Journal of Botany*, *1*(January 2008), 32–37.

K. R. Saravanan, K. S. and B. C. C. (2013). Important Coastal and Marine Biodiversity Areas of India(ICMBAs). *Coastal and Marine Protected Areas in India: Challenges and Way Forward, ENVIS Bulletin: Wildlife & Protected Areas., January 2013*. http://www.wiienvis.nic.in/Database/ICMBAs_8247.aspx

Kalasariya, H. S., Pereira, L., & Patel, N. B. (2023). Comprehensive Phytochemical Analysis

and Bioactivity Evaluation of Padina boergesenii: Unveiling Its Prospects as a Promising Cosmetic Component. *Marine Drugs*, *21*(7), 385. https://doi.org/10.3390/md21070385

Kamleshbhai, B. P., Joshi, N. H., & Tiwari, A. (2022). *Agri-India*. *May*.

Kang, Y., Deng, Z., Zang, R., & Long, W. (2017). DNA barcoding analysis and phylogenetic relationships of tree species in tropical cloud forests. *Scientific Reports*, *7*(1), 1–9. https://doi.org/10.1038/s41598-017-13057-0

Kannan, M., Pushparaj, A., Dheeba, B., Nageshwari, K., & Kannan, K. (2014). *Journal of Chemical and Pharmaceutical Research , 2014 , 6 (11): 312-318 Research Article Phytochemical screening and antioxidant activity of marine algae Gracilaria corticata and Spirulina platensis*. *6*(11), 312–318.

Kashtoh, H., & Baek, K. H. (2022). Recent Updates on Phytoconstituent Alpha-Glucosidase Inhibitors: An Approach towards the Treatment of Type Two Diabetes. *Plants*, *11*(20). https://doi.org/10.3390/plants11202722

Keshvari, S., Ceddia, R. P., Rajbhandari, P., Chaurasia, B., & Bond, S. T. (2022). Editorial: Adipose Tissue in Obesity and Metabolic Disease. *Frontiers in Physiology*, *13*(April), 1–2. https://doi.org/10.3389/fphys.2022.898861

Khan, Z., Gul, H., Rauf, M., Arif, M., Hamayun, M., Ud-Din, A., Sajid, Z. A., Khilji, S. A., Rehman, A., Tabassum, A., Parveen, Z., & Lee, I. J. (2022). Sargassum wightii Aqueous Extract Improved Salt Stress Tolerance in Abelmoschus esculentus by Mediating Metabolic and Ionic Rebalance. *Frontiers in Marine Science*, *9*(June), 1–19. https://doi.org/10.3389/fmars.2022.853272

Khaw, K. Y., Parat, M. O., Shaw, P. N., & Falconer, J. R. (2017). Solvent supercritical fluid technologies to extract bioactive compounds from natural sources: A review. *Molecules*, *22*(7). https://doi.org/10.3390/molecules22071186

Koller, R. L. (1957). Amplification. *Physics Today*, *10*(1), 34–36. https://doi.org/10.1063/1.3060231

Kulkarni, S. A., Krishnan, S. B. B., Chandrasekhar, B., Banerjee, K., Sohn, H., & Madhavan, T. (2021). Characterization of Phytochemicals in Ulva intestinalis L. and Their Action Against SARS-CoV-2 Spike Glycoprotein Receptor-Binding Domain. *Frontiers in Chemistry*, *9*(September), 1–16. https://doi.org/10.3389/fchem.2021.735768

Kumar, A. (2012). Maritime History of India: An Overview. *Maritime Affairs:Journal of the National Maritime Foundation of India*, *8*(1), 93–115. https://doi.org/10.1080/09733159.2012.690562

Kumar, A., Nirmal, P., Kumar, M., Jose, A., Tomer, V., Oz, E., Proestos, C., Zeng, M., Elobeid, T., Sneha, V., & Oz, F. (2023). Major Phytochemicals: Recent Advances in Health Benefits and Extraction Method. *Molecules*, *28*(2), 1–41. https://doi.org/10.3390/molecules28020887

Kumar, A., Semwal, R., Chauhan, A., Semwal, R. B., Chandra, S., Sircar, D., Roy, P., & Semwal, D. K. (2022). Evaluation of antidiabetic effect of Cissampelos pareira L. (Menispermaceae) root extract in streptozotocin-nicotinamide-induced diabetic rats via

targeting SGLT2 inhibition. *Phytomedicine Plus*, *2*(4), 100374. https://doi.org/10.1016/j.phyplu.2022.100374

Kusumastuti, A., Anis, S., & Fardhyanti, D. S. (2019). *Production of natural dyes powder based on chemo-physical technology for textile application Production of natural dyes powder based on chemo-physical technology for textile application*. https://doi.org/10.1088/1755-1315/258/1/012028

Kwon, Y. I. I., Vattem, D. A., & Shetty, K. (2006). Evaluation of clonal herbs of Lamiaceae species for management of diabetes and hypertension. *Asia Pacific Journal of Clinical Nutrition*, *15*(1), 107–118.

Lakshmanan, K., & Padmanabhan, S. (2022). Phytochemical analysis and antioxidant activity of seaweed extracts. *~ 527 ~ The Pharma Innovation Journal*, *11*(1), 527–531. http://www.thepharmajournal.com

Leandro, A., Monteiro, P., Pacheco, D., Figueirinha, A., Gonçalves, A. M. M., Jorge, G., & Pereira, L. (2020). Seaweed Phenolics: From Extraction to Applications. *Marine Drugs*, *18*(8), 1–47.

Leelavathi, M. S., & Prasad, M. P. (2014). Evaluation of Antioxidant properties of Marine Sea Weed samples by DPPH method. *International Journal Of Pure & Applied Bioscience*, *2*(6), 132–137. www.ijpab.com

Lemes, A. C., Egea, M. B., Oliveira Filho, J. G. de, Gautério, G. V., Ribeiro, B. D., & Coelho, M. A. Z. (2022). Biological Approaches for Extraction of Bioactive Compounds From Agro-industrial By-products: A Review. *Frontiers in Bioengineering and Biotechnology*, *9*(January), 1–18. https://doi.org/10.3389/fbioe.2021.802543

Li, C., Zha, W., Li, W., Wang, J., & You, A. (2023). Advances in the Biosynthesis of Terpenoids and Their Ecological Functions in Plant Resistance. *International Journal of Molecular Sciences*, *24*(14). https://doi.org/10.3390/ijms241411561

Liu, L., Heinrich, M., Myers, S., & Dworjanyn, S. A. (2012). Towards a better understanding of medicinal uses of the brown seaweed Sargassum in Traditional Chinese Medicine: A phytochemical and pharmacological review. *Journal of Ethnopharmacology*, *142*(3), 591–619. https://doi.org/10.1016/j.jep.2012.05.046

Liyanage, N. M., Lee, H. G., Nagahawatta, D. P., Jayawardhana, H. H. A. C. K., Song, K. M., Choi, Y. S., Jeon, Y. J., & Kang, M. C. (2023). Fucoidan from Sargassum autumnale Inhibits Potential Inflammatory Responses via NF-κB and MAPK Pathway Suppression in Lipopolysaccharide-Induced RAW 264.7 Macrophages. *Marine Drugs*, *21*(7). https://doi.org/10.3390/md21070374

Lomartire, S., & Gonçalves, A. M. M. (2022a). An Overview of Potential Seaweed-Derived Bioactive Compounds for Pharmaceutical Applications. In *Marine Drugs* (Vol. 20, Issue 2). MDPI. https://doi.org/10.3390/md20020141

Lomartire, S., & Gonçalves, A. M. M. (2022b). Novel Technologies for Seaweed Polysaccharides Extraction and Their Use in Food with Therapeutically Applications—A Review. *Foods*, *11*(17). https://doi.org/10.3390/foods11172654

Lopes, D., Rey, F., Leal, M. C., Lillebø, A. I., Calado, R., & Domingues, M. R. (2021).

Bioactivities of lipid extracts and complex lipids from seaweeds: Current knowledge and future prospects. *Marine Drugs*, *19*(12), 1–24. https://doi.org/10.3390/md19120686

Lordan, S., Smyth, T. J., Soler-Vila, A., Stanton, C., & Paul Ross, R. (2013). The α-amylase and α-glucosidase inhibitory effects of Irish seaweed extracts. *Food Chemistry*, *141*(3), 2170–2176. https://doi.org/10.1016/j.foodchem.2013.04.123

Lu, L. W., & Chen, J. H. (2022). Seaweeds as Ingredients to Lower Glycemic Potency of Cereal Foods Synergistically—A Perspective. *Foods*, *11*(5), 1–24. https://doi.org/10.3390/foods11050714

Lu, X., Dissanayake, A. A., Xiao, C., Gao, J., Zhao, M., & Nair, M. G. (2022). The edible seaweed Laminaria japonica contains cholesterol analogues that inhibit lipid peroxidation and cyclooxygenase enzymes. *PLoS ONE*, *17*(1 January), 1–14. https://doi.org/10.1371/journal.pone.0258980

Magalhaes, K. D., Costa, L. S., Fidelis, G. P., Oliveira, R. M., Nobre, L. T. D. B., Dantas-Santos, N., Camara, R. B. G., Albuquerque, I. R. L., Cordeiro, S. L., Sabry, D. A., Costa, M. S. S. P., Alves, L. G., & Rocha, H. A. O. (2011). Anticoagulant, antioxidant and antitumor activities of heterofucans from the seaweed dictyopteris delicatula. *International Journal of Molecular Sciences*, *12*(5), 3352–3365. https://doi.org/10.3390/ijms12053352

Mahendran, S., Maheswari, P., Sasikala, V., Rubika, J. jaya, & Pandiarajan, J. (2021). In vitro antioxidant study of polyphenol from red seaweeds dichotomously branched gracilaria Gracilaria edulis and robust sea moss Hypnea valentiae. *Toxicology Reports*, *8*(June 2020), 1404–1411. https://doi.org/10.1016/j.toxrep.2021.07.006

Mahmud, A. R., Ema, T. I., Siddiquee, M. F. R., Shahriar, A., Ahmed, H., Mosfeq-Ul-Hasan, M., Rahman, N., Islam, R., Uddin, M. R., & Mizan, M. F. R. (2023). Natural flavonols: actions, mechanisms, and potential therapeutic utility for various diseases. *Beni-Suef University Journal of Basic and Applied Sciences*, *12*(1). https://doi.org/10.1186/s43088-023-00387-4

Malhotra, R., C, T. S., & Cojandaraj, L. (n.d.). *Phytochemicals and Antioxidant Activities of Nitophyllum Marginale*.

Mallhi, I. Y., Sohaib, M., Khan, A. U., & Rabbani, I. (2023). *Antidiabetic, Antioxidative and Antihyperlipidemic Effects of Strawberry Fruit Extract in Alloxan-Induced Diabetic Rats*. 1–11.

Mandlik, R. V., Naik, S. R., Zine, S., Ved, H., & Doshi, G. (2022). Antidiabetic Activity of Caulerpa racemosa: Role of Proinflammatory Mediators, Oxidative Stress, and Other Biomarkers. *Planta Medica International Open*, *9*(01), e60–e71. https://doi.org/10.1055/a-1712-8178

Maneein, S., Milledge, J. J., Nielsen, B. V., & Harvey, P. J. (2018). A review of seaweed pre-treatment methods for enhanced biofuel production by anaerobic digestion or fermentation. *Fermentation*, *4*(4). https://doi.org/10.3390/fermentation4040100

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

https://doi.org/10.1006/bbrc.1994.1764

Marcovecchio, J. E., Buzzi, N. S., Tartara, M. N., Spetter, C. V., & Simonetti, P. (2013). Potential effects of climate changes on the marine ecosystem stability: Assessment of the water quality. *Marine Ecology in a Changing World*, *December*, 1–42. https://doi.org/10.1201/b16334-3

Marinho, G. S., Sørensen, A. D. M., Safafar, H., Pedersen, A. H., & Holdt, S. L. (2019). Antioxidant content and activity of the seaweed Saccharina latissima: a seasonal perspective. *Journal of Applied Phycology*, *31*(2), 1343–1354. https://doi.org/10.1007/s10811-018-1650-8

Marques, R. (2022). Controlling Light to Optimize Growth and Added Value of the Green Macroalga Codium tomentosum. *Frontiers in Marine Science*, *9*(June), 1–11. https://doi.org/10.3389/fmars.2022.906332

Marudamuthu, A. S. (2015). *Antidiabetic Effect of Boerhavia diffusa : Effect on Serum and Tissue Lipids in Experimental Diabetes*. *March*, 4–9. https://doi.org/10.1089/jmf.2004.7.472

Mata, T. M. (2021). *Microalgae Biomolecules: Extraction, Separation and Purification Methods*. 1–43.

Matboli, M., Kamel, M. M., Essawy, N., Bekhit, M. M., Abdulrahman, B., Mohamed, G. F., & Eissa, S. (2021). Article identification of novel insulin resistance related cerna network in t2dm and its potential editing by crispr/cas9. *International Journal of Molecular Sciences*, *22*(15), 1–16. https://doi.org/10.3390/ijms22158129

Mazreku, I. N., Ahmetaj, H., Aliko, V., Bislimi, K., Halili, F., & Halili, J. (2017). Activity of catalase (CAT), ALT and AST in different organs of swiss albino mice treated with lead acetate, vitamin C and magnesium-L-threonate. *Journal of Clinical and Diagnostic Research*, *11*(11), CC01–CC04. https://doi.org/10.7860/JCDR/2017/30297.10841

Mcdermott, M. T., & Trujillo, J. M. (2020). DIABETES MELLITUS : CLASSIFICATION , ETIOLOGY , AND PATHOGENESIS. In *Diabetes Secrets* (Vol. 2). INC. https://doi.org/10.1016/B978-0-323-79262-2.00001-9

MD FAHIM, INAMUR RAHMAN, NIDA NASEEM, NAZIA IMAM, HINA YOUNUS, HASEEB AHSAN, & SIDDIQUI, W. A. (2022). Antidiabetic potential of natural phytochemical antioxidants. *Journal of Chemistry and Nutritional Biochemistry*, *3*(2), 26–43. https://doi.org/10.48185/jcnb.v3i2.610

Meinita, M. D. N., Harwanto, D., & Choi, J. S. (2022). Seaweed Exhibits Therapeutic Properties against Chronic Diseases: An Overview. In *Applied Sciences (Switzerland)* (Vol. 12, Issue 5). https://doi.org/10.3390/app12052638

Melakhessou, M. A., Marref, S. E., Benkiki, N., Marref, C., Becheker, I., & Khattabi, L. (2021). In vitro, acute and subchronic evaluation of the antidiabetic activity of Atractylis flava Desf n-butanol extract in alloxan-diabetic rats. *Future Journal of Pharmaceutical Sciences*, *7*(1). https://doi.org/10.1186/s43094-021-00358-5

Menaa, F., Wijesinghe, U., Thiripuranathar, G., Althobaiti, N. A., Albalawi, A. E., Khan, B. A., & Menaa, B. (2021). Marine algae-derived bioactive compounds: A new wave of

nanodrugs? *Marine Drugs*, *19*(9), 1–36. https://doi.org/10.3390/md19090484

Menon, A., Ornthammarath, T., Corigliano, M., & Lai, C. G. (2010). Probabilistic seismic hazard macrozonation of Tamil Nadu in Southern India. *Bulletin of the Seismological Society of America*, *100*(3), 1320–1341. https://doi.org/10.1785/0120090071

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

Mohamed, S., Hashim, S. N., & Rahman, H. A. (2012). Seaweeds: A sustainable functional food for complementary and alternative therapy. *Trends in Food Science and Technology*, *23*(2), 83–96. https://doi.org/10.1016/j.tifs.2011.09.001

Mohan, G. K., Sravya, V., Mohan, K., & Srivani, A. (2023). Phytochemical and pharmacological properties of Halimeda gracilis (Marine seaweed): A Review. *~ 125 ~ Journal of Pharmacognosy and Phytochemistry*, *12*(1), 125–129. https://www.phytojournal.com

Mohapatra, L., Bhattamisra, S. K., Panigrahy, R. C., & Parida, S. (2018). Evaluation of antioxidant, hypoglycaemic, antidiabetic and alpha amylase inhibiting activities of Sargassum wightii collected from the east coast of India. *Indian Drugs*, *55*(1), 61–69. https://doi.org/10.53879/id.55.01.10988

Mohy El-Din, S. M., & El-Ahwany, A. M. D. (2016). Bioactivity and phytochemical constituents of marine red seaweeds (Jania rubens , Corallina mediterranea and Pterocladia capillacea) . *Journal of Taibah University for Science*, *10*(4), 471–484. https://doi.org/10.1016/j.jtusci.2015.06.004

Mokhena, T., Mochane, M., Tshwafo, M., Linganiso, L., Thekisoe, O., & Songca, S. (2016). We are IntechOpen , the world ' s leading publisher of Open Access books Built by scientists , for scientists TOP 1 %. *Intech*, 225–240. https://www.intechopen.com/books/advanced-biometric-technologies/liveness-detection-in-biometrics

Movahhedin, N., Barar, J., Azad, F. F., Barzegari, A., & Nazemiyeh, H. (2014). Phytochemistry and biologic activities of caulerpa peltata native to Oman sea. *Iranian Journal of Pharmaceutical Research*, *13*(2), 515–521.

Mshiywa, F. M., Edwards, S., & Bradley, G. (2023). Rhodophyta DNA Barcoding: Ribulose-1, 5-Bisphosphate Carboxylase Gene and Novel Universal Primers. *International Journal of Molecular Sciences*, *25*(1), 58. https://doi.org/10.3390/ijms25010058

MultiLab. (2003). *Soxhlet Extraction Apparatus*. *July*, 0–4. http://www.multilab.biz/laboratory-glasswares/extraction/x89-soxhlet-extraction-apparatus.htm

Murakami, K., Okajima, K., & Okabe, H. (1995). Catalase. *Nippon Rinsho. Japanese Journal of Clinical Medicine*, *53 Su Pt 1*, 358–360. https://doi.org/10.1086/330448

Naik, Y. K., Sharma, V., Arora, S., & Seth, R. (2023). Development and application of DPPH impregnated paper based color sensor disc to detect vegetable oils addition in cow ghee. *Journal of Food Science and Technology*, *60*(12), 3014–3023. https://doi.org/10.1007/s13197-023-05830-z

Naveen, J., Baskaran, R., & Baskaran, V. (2021). Profiling of bioactives and in vitro evaluation of antioxidant and antidiabetic property of polyphenols of marine algae Padina tetrastromatica. *Algal Research*, *55*(February), 102250. https://doi.org/10.1016/j.algal.2021.102250

Naz, D., Muhamad, A., Zeb, A., & Shah, I. (2019). In vitro and in vivo Antidiabetic Properties of Phenolic Antioxidants From Sedum adenotrichum. *Frontiers in Nutrition*, *6*(November), 1–7. https://doi.org/10.3389/fnut.2019.00177

Ni, L., Yuan, C., Chen, G., Chen, G., Zhang, C., Zhang, C., Zhang, C., Zhang, C., & Wu, X. (2020). SGLT2i: Beyond the glucose-lowering effect. *Cardiovascular Diabetology*, *19*(1), 1–10. https://doi.org/10.1186/s12933-020-01071-y

Nie, T., & Cooper, G. J. S. (2021). Mechanisms Underlying the Antidiabetic Activities of Polyphenolic Compounds: A Review. *Frontiers in Pharmacology*, *12*(December), 1–27. https://doi.org/10.3389/fphar.2021.798329

Noeman, S. A., Hamooda, H. E., & Baalash, A. A. (2011). Biochemical study of oxidative stress markers in the liver, kidney and heart of high fat diet induced obesity in rats. *Diabetology and Metabolic Syndrome*, *3*(1), 1–8. https://doi.org/10.1186/1758-5996-3-17

Obstetric analgesia and anesthesia. (1996). In *International Journal of Gynecology and Obstetrics* (Vol. 54, Issue 3, pp. 281–292). https://doi.org/10.1016/S0020-7292(96)90087-9

Odeyemi, S., & Dewar, J. (2020). In vitro antidiabetic activity affecting glucose uptake in HepG2 cells following their exposure to extracts of Lauridia tetragona (L.f.) R.H. Archer. *Processes*, *8*(1). https://doi.org/10.3390/pr8010033

Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*, *95*(2), 351–358. https://doi.org/10.1016/0003-2697(79)90738-3

Osigwe, C. C., Akah, P. A., & Nworu, C. S. (2017). Biochemical and Haematological Effects of the Leaf Extract of <i>Newbouldia laevis</i> in Alloxan-Induced Diabetic Rats. *Journal of Biosciences and Medicines*, *05*(06), 18–36. https://doi.org/10.4236/jbm.2017.56003

P, A. P., & J, J. P. P. (2022). *ORIGINAL ARTICLE Phytochemical and molecular docking analysis of the ethanolic extract of Codium decorticatum (Woodward) M . Howe .* *11*(September), 16–23.

P, C. G. T., & R, S. K. (2015). Research Article Phytochemical examination , antioxidant potential and in vitro antibacterial studies of crude extracts of Parthenium hysterophorus Linn . leaves. *J.Chem.Pharm.Res.*, *7*(4), 219–225.

Palumbi, S. R., Sandifer, P. A., Allan, J. D., Beck, M. W., Fautin, D. G., Fogarty, M. J., Halpera, B. S., Incze, L. S., Leong, J. A., Norse, E., Stachowicz, J. J., & Wall, D. H. (2009). Managing for ocean biodiversity to sustain marine ecosystem services. *Frontiers in Ecology and the Environment*, *7*(4), 204–211. https://doi.org/10.1890/070135

Pan, Y., Wu, L. J., & Yu, Z. L. (2006). Effect of salt and drought stress on antioxidant enzymes activities and SOD isoenzymes of liquorice (Glycyrrhiza uralensis Fisch). *Plant Growth Regulation*, *49*(2–3), 157–165. https://doi.org/10.1007/s10725-006-9101-y

Pandey, A. K., Chauhan, O. P., & Semwal, A. D. (2020). Seaweeds – a potential source for functional foods. *Defence Life Science Journal*, *5*(4), 315–322. https://doi.org/10.14429/dlsj.5.15632

Pandey, G., Chatterjee, N. S., Panda, S. K., Mohan, C. O., Kishore, P., Kumar, A., Uchoi, D., Balasundari, S., Anandan, R., Mathew, S., & Ravishankar, C. N. (2023a). Scope and challenges of seaweed utilization in food and nutraceutical industry in India: a review. *Journal of Food Science and Technology*. https://doi.org/10.1007/s13197-023-05676-5

Pandey, G., Chatterjee, N. S., Panda, S. K., Mohan, C. O., Kishore, P., Kumar, A., Uchoi, D., Balasundari, S., Anandan, R., Mathew, S., & Ravishankar, C. N. (2023b). Scope and challenges of seaweed utilization in food and nutraceutical industry in India: a review. *Journal of Food Science and Technology*, *61*(2), 230–241. https://doi.org/10.1007/s13197-023-05676-5

Pérez-Lloréns, J. L., Critchley, A. T., Cornish, M. L., & Mouritsen, O. G. (2023). Saved by seaweeds (II): Traditional knowledge, home remedies, medicine, surgery, and pharmacopoeia. *Journal of Applied Phycology*, *35*(5), 2049–2068. https://doi.org/10.1007/s10811-023-02965-6

Peroxides, O., & Marquis, P. E. I. (1978). *United States Patent (19)*. *19*, 1–6.

Pharmawati, M., Basyar, W. M., & Astarini, I. A. (2020). Total Genomic DNA Extraction Studies from Seaweeds. *Advances in Tropical Biodiversity and Environmental Sciences*, *4*(1), 10. https://doi.org/10.24843/atbes.2020.v04.i01.p03

Piko, P., Werissa, N. A., Fiatal, S., Sandor, J., & Adany, R. (2021). Impact of genetic factors on the age of onset for type 2 diabetes mellitus in addition to the conventional risk factors. *Journal of Personalized Medicine*, *11*(1), 1–17. https://doi.org/10.3390/jpm11010006

Praba, N., & Sumaya, D. S. (2022). Study On Phytochemical and Antioxidant Properties of Padina gymnospora and Ulva lactuca. *International Journal of Life Science and Pharma Research*, *12*(6), 155–160. https://doi.org/10.22376/ijpbs/lpr.2022.12.6.l155-160

Prajapati, V. D., Maheriya, P. M., Jani, G. K., & Solanki, H. K. (2014). Carrageenan: A natural seaweed polysaccharide and its applications. In *Carbohydrate Polymers* (Vol. 105, Issue 1). Elsevier Ltd. https://doi.org/10.1016/j.carbpol.2014.01.067

Premarathna, A. D., Ranahewa, T. H., Wijesekera, S. K., Harishchandra, D. L., Karunathilake, K. J. K., Waduge, R. N., Wijesundara, R. R. M. K. K., Jayasooriya, A. P., Wijewardana, V., & Rajapakse, R. P. V. J. (2020). Preliminary screening of the aqueous extracts of twenty-three different seaweed species in Sri Lanka with in-vitro and in-vivo assays. *Heliyon*, *6*(6), e03918. https://doi.org/10.1016/j.heliyon.2020.e03918

Prizomwala, S. P., Tandon, A., Kazmer, M., Makwana, N., Solanki, T., & Chauhan, G. (2022). Geoheritage Potential of Miliolite Formation of the Southern Saurashtra (Western India), Gujarat. *Geoheritage*, *14*(4). https://doi.org/10.1007/s12371-022-

00761-1

Professional Practice Committee: Standards of Medical Care in Diabetes-2020. (2020). *Diabetes Care*, *43*(January), S3. https://doi.org/10.2337/dc20-Sppc

Qin, S., Liu, H., Nie, Z., Gao, W., Li, C., Lin, Y., & Zhao, P. (2018). AsA–GSH Cycle and Antioxidant Enzymes Play Important Roles in Cd Tolerance of Wheat. *Bulletin of Environmental Contamination and Toxicology*, *101*(5), 684–690. https://doi.org/10.1007/s00128-018-2471-9

R, H. V. P. U., Centre, D., & Nadu, T. (2017). *INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH EVALUATION OF PHYTOCHEMICAL CONSTITUENTS OF TWO DIFFERENT SPECIES OF TURBINARIA Biochemistry KEYWORDS : 7*, 224–226.

Rachdaoui, N. (2020). Insulin: The friend and the foe in the development of type 2 diabetes mellitus. *International Journal of Molecular Sciences*, *21*(5), 1–21. https://doi.org/10.3390/IJMS21051770

Rahman, F., Abdul, A., Ahsan, T., Islam, T., Alam, M., & Hossain, M. N. (2021). Screening of Potential Bioactive Compounds from Padina gymnospora Found In the Coast Of St. Martin Island Of Bangladesh. *Journal of Marine Biology and Aquaculture*, *6*(1), 1–7. https://doi.org/10.15436/2381-0750.21.3010

Rajkumar, G., Saravana, P., & Srinivasan, V. (2017). *Phytochemical Screenings of the Marine Red Alga , Gracilaria Corticata*. *01*(08), 90–97.

Ramaswamy, R. S., Prathyusha, N., Saranya, R., Sumathy, H., Mohanavalli, K. T., Priya, R. J., Venkhatesh, J. R., Babu, C. S., Manickavasakam, K., & Thanikachalam, S. (2012). Acute toxicity and the 28-day repeated dose study of a Siddha medicine Nuna Kadugu in rats. *BMC Complementary and Alternative Medicine*, *12*. https://doi.org/10.1186/1472-6882-12-190

Ramatchandirin, B., Pearah, A., & He, L. (2023). Regulation of Liver Glucose and Lipid Metabolism by Transcriptional Factors and Coactivators. *Life*, *13*(2), 1–23. https://doi.org/10.3390/life13020515

Ramawat, K. G., & Mérillon, J. M. (2013). Natural products: Phytochemistry, botany and metabolism of alkaloids, phenolics and terpenes. *Natural Products: Phytochemistry, Botany and Metabolism of Alkaloids, Phenolics and Terpenes*, 1–4242. https://doi.org/10.1007/978-3-642-22144-6

Ramdani, M., Elasri, O., Saidi, N., Elkhiati, N., Taybi, F. A., Mostareh, M., Zaraali, O., Haloui, B., & Ramdani, M. (2017). Evaluation of antioxidant activity and total phenol content of Gracilaria bursa-pastoris harvested in Nador lagoon for an enhanced economic valorization. *Chemical and Biological Technologies in Agriculture*, *4*(1), 1–7. https://doi.org/10.1186/s40538-017-0110-z

Ramesh, C. H., Koushik, S., Shunmugaraj, T., & Murthy, M. V. R. (2020). Current biodiversity of Mandapam group of Islands in Gulf of Mannar Marine Biosphere Reserve , Southeast coast of Tamil Nadu , India. *Regional Studies in Marine Science*, *39*, 101429. https://doi.org/10.1016/j.rsma.2020.101429

Ranjith, H. V., Sagar, D., Kalia, V. K., Dahuja, A., & Subramanian, S. (2023). Differential Activities of Antioxidant Enzymes, Superoxide Dismutase, Peroxidase, and Catalase vis-à-vis Phosphine Resistance in Field Populations of Lesser Grain Borer (Rhyzopertha dominica) from India. *Antioxidants*, *12*(2). https://doi.org/10.3390/antiox12020270

Rasyid, A. (2017). Evaluation of nutritional composition of the dried seaweed Ulva lactuca from Pameungpeuk waters, Indonesia. *Tropical Life Sciences Research*, *28*(2), 119–125. https://doi.org/10.21315/tlsr2017.28.2.9

Remya, R. R., Julius, A., Ramadoss, R., Parthiban, S., Bharath, N., Pavana, B., Samrot, A. V., Kanwal, S., Vinayagam, M., & Gemeda, F. W. (2022). Pharmacological Activities of Natural Products from Marine Seaweed Turbinaria ornata: A Review. In *Journal of Nanomaterials* (Vol. 2022). Hindawi Limited. https://doi.org/10.1155/2022/4784608

Rengasamy, K. R., Mahomoodally, M. F., Aumeeruddy, M. Z., Zengin, G., Xiao, J., & Kim, D. H. (2020). Bioactive compounds in seaweeds: An overview of their biological properties and safety. *Food and Chemical Toxicology*, *135*(September), 111013. https://doi.org/10.1016/j.fct.2019.111013

Retnamma, J., Kalathil, B. K., Loganathan, J., & Chinnadurai, K. (2021). *Why the Gulf of Mannar is a marine biological paradise ?* 64892–64907.

Review, P. (2014). Screening of phytochemicals and antimicrobial activity of Caulerpa scalpelliformis collected from Manapad Coast, Tuticorin District, Tamilnadu, South India. *Journal of Coastal Life Medicine*, *2*(2), 107–111. https://doi.org/10.12980/jclm.2.2014c1156

Rey, F., Cartaxana, P., Melo, T., Calado, R., Pereira, R., Abreu, H., Domingues, P., Cruz, S., & Rosário Domingues, M. (2020). Domesticated populations of Codium tomentosum display lipid extracts with lower seasonal shifts than conspecifics from the wild-relevance for biotechnological applications of this green seaweed. *Marine Drugs*, *18*(4). https://doi.org/10.3390/md18040188

Riaz, S. (2009). Diabetes mellitus. *Scientific Research and Essays*, *4*(5), 367–373.

Ribeiro, T. B., Melo, A., Vilas-Boas, A. A., & Pintado, M. (2023). Flavonoids. *Natural Secondary Metabolites: From Nature, Through Science, to Industry*, *2013*, 73–105. https://doi.org/10.1007/978-3-031-18587-8_4

Rogel-Castillo, C., Latorre-Castañeda, M., Muñoz-Muñoz, C., & Agurto-Muñoz, C. (2023). Seaweeds in Food: Current Trends. *Plants*, *12*(12), 1–19. https://doi.org/10.3390/plants12122287

Rout, S., Rath, B., Kumar, A., Kumar Bhattamisra, S., & Panda, J. (2020). Preliminary Phytochemical Analysis of the Crude Extract of Sargassum Wightii. *IJSDR2011034 International Journal of Scientific Development and Research*, *5*(11), 227–230.

Rubio-Cabezas, O., Hattersley, A. T., Njølstad, P. R., Mlynarski, W., Ellard, S., White, N., Chi, D. V., & Craig, M. E. (2014). The diagnosis and management of monogenic diabetes in children and adolescents. *Pediatric Diabetes*, *15*(SUPPL.20), 47–64. https://doi.org/10.1111/pedi.12192

Ruch, R. J., Cheng, S. jun, & Klaunig, J. E. (1989). Prevention of cytotoxicity and inhibition

of intercellular communication by antioxidant catechins isolated from chinese green tea. *Carcinogenesis*, *10*(6), 1003–1008. https://doi.org/10.1093/carcin/10.6.1003

Rushdi, M. I., Abdel-Rahman, I. A. M., Attia, E. Z., Saber, H., Saber, A. A., Bringmann, G., & Abdelmohsen, U. R. (2022). The Biodiversity of the Genus Dictyota: Phytochemical and Pharmacological Natural Products Prospectives. *Molecules*, *27*(3), 1–30. https://doi.org/10.3390/molecules27030672

Sahoo, D. R., & Biswal, T. (2021). Alginate and its application to tissue engineering. *SN Applied Sciences*, *3*(1), 1–19. https://doi.org/10.1007/s42452-020-04096-w

Samar, J., Butt, G. Y., Shah, A. A., Shah, A. N., Ali, S., Jan, B. L., Abdelsalam, N. R., & Hussaan, M. (2022). Phycochemical and Biological Activities From Different Extracts of Padina antillarum (Kützing) Piccone. *Frontiers in Plant Science*, *13*(July), 1–18. https://doi.org/10.3389/fpls.2022.929368

Santhanam, R. C., Ali, S., Yahoob, M., & Venkatraman, A. (2019). *Anti Scavenging Property And Characterization Using HPLC Of The Fucoidan extracted from*. *8*(11), 2201–2206.

Santhanam, R. C., Yacoob, S. A. M., & Venkatraman, A. (2022). Invitro cytotoxicity assay of Fucoidan extracted from Turbinaria conoides against cancer cell lines MCF7, A549, and normal cell line L929. *Brazilian Journal of Pharmaceutical Sciences*, *58*, 1–6. https://doi.org/10.1590/s2175-97902022e19542

Saptarini, N. M., & Herawati, I. E. (2019). The colorimetric method for determination of total Alkaloids and Flavonoids content in Indonesian black nightshade (Solanum nigrum L.). *Journal of Advanced Pharmacy Education and Research*, *9*(3), 80–84.

Sathya, R., Kanaga, N., Sankar, P., & Jeeva, S. (2017). Antioxidant properties of phlorotannins from brown seaweed Cystoseira trinodis (Forsskål) C. Agardh. *Arabian Journal of Chemistry*, *10*, S2608–S2614. https://doi.org/10.1016/j.arabjc.2013.09.039

Senthilkumar, D., & Jayanthi, S. (2015). Antioxidant activities of purified glycoprotein extracted from Codium decorticatum. *Journal of Applied Pharmaceutical Science*, *5*(12), 101–104. https://doi.org/10.7324/JAPS.2015.501217

Series, C. (2019). *Total phenolic , antioxidant activity and toxicity effect of Turbinaria decurrens extracts from South Sulawesi*. https://doi.org/10.1088/1742-6596/1341/3/032008

Shah, N. A., & Khan, M. R. (2014). Antidiabetic effect of Sida cordata in alloxan induced diabetic rats. *BioMed Research International*, *2014*. https://doi.org/10.1155/2014/671294

Shah, N. J. (2019). Polymerase chain reaction. *Introduction to Basics of Pharmacology and Toxicology: Volume 1: General and Molecular Pharmacology: Principles of Drug Action*, *2*(3), 395–397. https://doi.org/10.1007/978-981-32-9779-1_31

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

Sharifuddin, Y., Chin, Y. X., Lim, P. E., & Moi, S. (2015). *Potential Bioactives from Seaweed for Diabetes Management*. *4*, 5447–5491. https://doi.org/10.3390/md13085447

Shen, S., Li, Y., Wu, X., & Ding, L. (2010). Sequences and phylogeny analysis of rbcL gene in marine Chlorophyta. *Journal of Ocean University of China*, *9*(2), 145–150. https://doi.org/10.1007/s11802-010-0145-2

Silva-Brito, F., Pereira, S. G., Rocha, C. M. R., da Costa, E., Domingues, M. R., Azevedo, A., Kijjoa, A., Abreu, H., & Magnoni, L. (2021). Improving agar properties of farmed Gracilaria gracilis by using filtered sunlight. *Journal of Applied Phycology*, *33*(5), 3397–3411. https://doi.org/10.1007/s10811-021-02497-x

Silva, D., Bambaranda, B. V. A. S. ., & Mudannayake, D. C. (2022). Bioactive, Microbiological, and Sensory Properties of Sargassum cristaefolium and Sargassum crassifolium Herbal Tea. *Advances in Technology*, *2*(1), 38–49. https://doi.org/10.31357/ait.v2i1.5456

Silva, G., Gunathilaka, T. L., Samarakoon, K. W., Ranasinghe, P., & Peiris, L. D. C. (2019). *and Identification of Bioactive Compounds in Phenol-Rich Extract from the Marine Red Algae*. 1–17.

Singh, B. K., Chopra, R. C., Rai, S. N., Verma, M. P., & Mohanta, R. K. (2016). Effect of Feeding Seaweed as Mineral Source on Mineral Metabolism, Blood and Milk Mineral Profile in Cows. *Proceedings of the National Academy of Sciences India Section B - Biological Sciences*, *86*(1), 89–95. https://doi.org/10.1007/s40011-014-0413-9

Siratantri, T., Yogabuana, M., & Olivia, S. (2014). *An In Vitro Study of Antidiabetic Activity of Sargassum Duplicatum and Turbinaria Decurens Seaweed*. *3*(2), 13–18.

Smyth, P. P. A. (2021). Iodine, Seaweed, and the Thyroid. *European Thyroid Journal*, *10*(2), 101–108. https://doi.org/10.1159/000512971

Soares, C., Sousa, S., Machado, S., Vieira, E., Carvalho, A. P., Ramalhosa, M. J., Morais, S., Correia, M., Oliva-Teles, T., Domingues, V. F., & Delerue-Matos, C. (2021). Bioactive lipids of seaweeds from the portuguese north coast: Health benefits versus potential contamination. *Foods*, *10*(6), 1–13. https://doi.org/10.3390/foods10061366

Sofowora, A., Ogunbodede, E., & Onayade, A. (2013). The role and place of medicinal plants in the strategies for disease prevention. *African Journal of Traditional, Complementary, and Alternative Medicines : AJTCAM / African Networks on Ethnomedicines*, *10*(5), 210–229. https://doi.org/10.4314/ajtcam.v10i5.2

Soomro, S. (2019). Oxidative Stress and Inflammation. *Open Journal of Immunology*, *09*(01), 1–20. https://doi.org/10.4236/oji.2019.91001

Sørensen, L. E., Jeppesen, P. B., Christiansen, C. B., Hermansen, K., & Gregersen, S. (2019). Nordic seaweed and diabetes prevention: Exploratory studies in KK-Ay mice. *Nutrients*, *11*(6). https://doi.org/10.3390/nu11061435

Spatoglossum, S. (2020). *Different Bioactive Constituents and Biochemical Composition of Brown ISSN NO : 0042-9945 Page No : 349*. *June*.

Subbulakshmi, H. M. and A. P. C., Phytochemistry, P. and T. of M. P., & Lubna Tariq, Basharat Ahmad Bhat, S. S. H. & R. A. M. (2018). Phytochemistry, Pharmacology and Toxicity of Medicinal Plants. *Springer*.

Subha, V. (2020). *Antidiabetic Activity of Red Marine Algae In Vitro : A Review Antidiabetic*

Activity of Red Marine Algae In Vitro : A Review. November.

Sudarwati, W., Hardjomidjojo, H., Machfud, & Setyaningsih, D. (2020). Literature review: Potential and opportunities for the development of seaweed agro-industry. *IOP Conference Series: Earth and Environmental Science*, *472*(1). https://doi.org/10.1088/1755-1315/472/1/012063

Surendran, P. S. L., Ranjith, B. S. D. S., & Kumar, S. (2019). Facile green synthesis of gold nanoparticles from marine algae Gelidiella acerosa and evaluation of its biological Potential. *SN Applied Sciences*, *1*(4), 1–12. https://doi.org/10.1007/s42452-019-0284-z

Sutrisna, R. J., Rafiq, S., Sharma, A., Noor, T., Raffi, U., Iqbal, N., & Thayil, R. (n.d.). *Natural Dyes Extraction Intended for Coloring Process in Fashion Indutries Natural Dyes Extraction Intended for Coloring Process in Fashion Indutries*. https://doi.org/10.1088/1757-899X/833/1/012093

Suzuki, K., & Kono, T. (1980). Evidence that insulin causes translocation of glucose transport activity to the plasma membrane from an intracellular storage site. *Proceedings of the National Academy of Sciences of the United States of America*, *77*(5 I), 2542–2545. https://doi.org/10.1073/pnas.77.5.2542

Syad, A. N., Rajamohamed, B. S., Pandian, K., & Kasi, P. D. (2016). *Neuroprotective effect of the marine macroalga Gelidiella acerosa : identification of active compounds through bioactivity-guided fractionation*. *0209*. https://doi.org/10.3109/13880209.2016.1145700

Taenzer, L., Toth, G., & Hansel, C. M. (2024a). Assessment of hydrogen peroxide as a bioindicator of stress in seaweed aquaculture. *Scientific Reports*, *14*(1), 1–13. https://doi.org/10.1038/s41598-024-52182-5

Taenzer, L., Toth, G., & Hansel, C. M. (2024b). Assessment of hydrogen peroxide as a bioindicator of stress in seaweed aquaculture. *Scientific Reports*, *14*(1), 1–12. https://doi.org/10.1038/s41598-024-52182-5

Tekin, S., & Seven, E. (2022). Assessment of serum catalase, reduced glutathione, and superoxide dismutase activities and malondialdehyde levels in keratoconus patients. *Eye (Basingstoke)*, *36*(10), 2062–2066. https://doi.org/10.1038/s41433-021-01753-1

Tests, D., & Diabetes, F. O. R. (2016). 2. Classification and diagnosis of diabetes. *Diabetes Care*, *39*(January), S13–S22. https://doi.org/10.2337/dc16-S005

Thamada, S., Kumaran, S., & Vijayaraj, R. (2019). In vivo and In vitro Models for Biological Screening of Anti-Diabetic Drugs. *Article in International Journal of Pharmacy and Biological Sciences*, *9*(2), 294–298. https://doi.org/10.21276/ijpbs.2019.9.2.39

Thennarasan, S. S. M. V. S. A. J. of P. and. (n.d.). *Antidiabetic efficacy of Methanol extract Brown Alga Lobophora variegata on Alloxan stimulated Hyperglycemic Wistar Albino Rats.*

Theuerkauf, S. J., Barrett, L. T., Alleway, H. K., Costa-Pierce, B. A., St. Gelais, A., & Jones, R. C. (2022). Habitat value of bivalve shellfish and seaweed aquaculture for fish and invertebrates: Pathways, synthesis and next steps. *Reviews in Aquaculture*, *14*(1), 54–72. https://doi.org/10.1111/raq.12584

Thiebaud, D., Jacot, E., Maeder, E., & Felber, J. P. (1982). Effect of insulin on total glucose

uptake, glucose oxidation, and glucose storage in man. *Diabetes*, *31*(2 II Suppl. 2), 957–963.

Thulé, P. M. (2012). Mechanisms of current therapies for diabetes mellitus type 2. *American Journal of Physiology - Advances in Physiology Education*, *36*(4), 275–283. https://doi.org/10.1152/advan.00094.2012

Thurstan, R. H., Brittain, Z., Jones, D. S., Cameron, E., Dearnaley, J., & Bellgrove, A. (2018). Aboriginal uses of seaweeds in temperate Australia: an archival assessment. *Journal of Applied Phycology*, *30*(3), 1821–1832. https://doi.org/10.1007/s10811-017-1384-z

Tibbetts, S. M., Milley, J. E., & Lall, S. P. (2016). Nutritional quality of some wild and cultivated seaweeds: Nutrient composition, total phenolic content and in vitro digestibility. *Journal of Applied Phycology*, *28*(6), 3575–3585. https://doi.org/10.1007/s10811-016-0863-y

Trigo, J. P., Palmnäs-Bédard, M., Juanola, M. V. L., & Undeland, I. (2023). Effects of whole seaweed consumption on humans: current evidence from randomized-controlled intervention trials, knowledge gaps, and limitations. *Frontiers in Nutrition*, *10*(July). https://doi.org/10.3389/fnut.2023.1226168

Unnikrishnan, P. S., Animish, A., Madhumitha, G., Suthindhiran, K., & Jayasri, M. A. (2022). Bioactivity Guided Study for the Isolation and Identification of Antidiabetic Compounds from Edible Seaweed—Ulva reticulata. *Molecules*, *27*(24). https://doi.org/10.3390/molecules27248827

Unnikrishnan, P., Suthindhiran, K., & Jayasri, M. (2015). Alpha-amylase inhibition and antioxidant activity of marine green algae and its possible role in diabetes management. *Pharmacognosy Magazine*, *11*(44), 511. https://doi.org/10.4103/0973-1296.172954

Usman, A., Abdulrahman, F. I., & Usman, A. (2009). Qualitative phytochemical screening and in vitro antimicrobial effects of methanol stem bark extract of Ficus thonningii (Moraceae). *African Journal of Traditional, Complementary and Alternative Medicines*, *6*(3), 289–295. https://doi.org/10.4314/ajtcam.v6i3.57178

Uusitupa, M. (2002). Lifestyles matter in the prevention of type 2 diabetes. *Diabetes Care*, *25*(9), 1650–1651. https://doi.org/10.2337/diacare.25.9.1650

V. Sornalakshmi, P.S. Tresina, & K. Jeba Ananth. (2021). View of Phytochemical analysis and DPPH radical scavenging activity of marine red seaweed Gracilaria corticata. *Nat. Volatiles & Essent. Oils*, *8*(5), 3724–3730.

Vasquez, J. A., & Guerra, N. (1996). The use of seaweeds as bioindicators of natural and anthropogenic contaminants in northern Chile. *Hydrobiologia*, *326–327*(Table 1), 327–333. https://doi.org/10.1007/BF00047826

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

Vidyashree, J. S., Shetti, P. P., & Ghagane, S. C. (2024). Seaweeds as a potential resource in diabetes management: a review. *Future Journal of Pharmaceutical Sciences*, *10*(1). https://doi.org/10.1186/s43094-024-00583-8

Vijay Kumar & Kiran Dip Gill. (n.d.). Estimation of Blood Glucose Levels by Glucose Oxidase Method. *Basic Concepts in Clinical Biochemistry: A Practical Guide Pp 57–60*. https://doi.org/https://doi.org/10.1007/978-981-10-8186-6_13

Vinodkumar, M., Saravana, A., & Packirisamy, B. (2023). Effective Isolation of Brown Seaweed Flavonoids with Their Potential to Inhibit Free Radicals and Proliferative Cells. *Journal of Inorganic and Organometallic Polymers and Materials*, *33*(12), 3794–3804. https://doi.org/10.1007/s10904-023-02738-1

Viswanathan, S., Ebciba, C., & Nallamuthu, S. R. T. (2014). Phytochemical Screening and In Vitro Antibacterial , Antioxidant and Anticancer Activity of Amphiroa Fragilissima (Linneaus) J V Lamoroux. *International Journal of Innovative Research in Science, Engineering and Technology*, *3*(5), 12933–12948.

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. *International Journal Of Pharmaceutical Sciences And Research*, *4*(43), 2770–2776. https://doi.org/10.13040/IJPSR.0975-8232.4(7).2770-76

Wang, X., Zheng, P., Huang, G., Yang, L., & Zhou, Z. (2018). Dipeptidyl peptidase-4(DPP-4) inhibitors: promising new agents for autoimmune diabetes. *Clinical and Experimental Medicine*, *18*(4), 473–480. https://doi.org/10.1007/s10238-018-0519-0

Wang, Y., Ma, Y., Tao, L., Zhang, X., Hao, F., Zhao, S., Han, L., & Bai, C. (2022). Recent Advances in Separation and Analysis of Saponins in Natural Products. *Separations*, *9*(7). https://doi.org/10.3390/separations9070163

Wastewater, H. M., Znad, H., Awual, R., & Martini, S. (2022). *The Utilization of Algae and Seaweed Biomass for*. 1–21.

Watanabe, J., Kawabata, J., Kurihara, H., & Niki, R. (1997). Isolation and identification of α-glucosidase inhibitors from tochu-cha (eucommia ulmoides). *Bioscience, Biotechnology and Biochemistry*, *61*(1), 177–178. https://doi.org/10.1271/bbb.61.177

Webber, S. (2013). International Diabetes Federation. In *Diabetes Research and Clinical Practice* (Vol. 102, Issue 2). https://doi.org/10.1016/j.diabres.2013.10.013

Wei Yang a c, Zhifei Dou a, Yangxian Liu a, Yongchun Zhao b, R. H. c. (2023). Gaseous mercury capture using seaweed biochars modified by clean ultraviolet/hydrogen peroxide advanced oxidation process. *Journal of Cleaner Production Volume 389, 20 February 2023, 136121*, *389*(136121).

WEST, M., & ZIMMERMAN, H. J. (1959). Serum enzymes in hepatic disease. *The Medical Clinics of North America*, *43*(2), 371–400. https://doi.org/10.1016/S0025-7125(16)34158-X

Westman, E. C. (2021). Type 2 Diabetes Mellitus: A Pathophysiologic Perspective. *Frontiers in Nutrition*, *8*(August), 1–5. https://doi.org/10.3389/fnut.2021.707371

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). https://doi.org/10.1186/s43014-023-00132-5

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.*

Yamauchi, M., Kitamura, Y., Nagano, H., Kawatsu, J., & Gotoh, H. (2024). *DPPH Measurements and Structure — Activity Relationship Studies on the Antioxidant Capacity of Phenols*.

Yue, Q., Wang, Z., Tang, X., Zhao, C., Li, K., Su, L., Zhang, S., Sun, X., Liu, X., & Zhao, L. (2021). Hypolipidemic Effects of Fermented Seaweed Extracts by Saccharomyces cerevisiae and Lactiplantibacillus plantarum. *Frontiers in Microbiology*, *12*(November), 1–8. https://doi.org/10.3389/fmicb.2021.772585

Yulianti, E., Sri, M., & Wahyuningsih, H. (2022). *The effect of Kappaphycus alvarezii active fraction on oxidative stress and inflammation in streptozotocin and nicotinamide-induced diabetic rats*. *8*, 1–8. https://doi.org/10.1186/s12906-021-03496-8

Zaatout, H., Ghareeb, D., Abd-Elgwad, A., & Ismael, A. (2019). Phytochemical, antioxidant, and anti-inflammatory screening of the Egyptian Ulva lactuca methanolic extract. *Records of Pharmaceutical and Biomedical Sciences*, *0*(0), 33–38. https://doi.org/10.21608/rpbs.2019.12251.1032

Zang, L., Baharlooeian, M., Terasawa, M., Shimada, Y., & Nishimura, N. (2023). Beneficial effects of seaweed-derived components on metabolic syndrome via gut microbiota modulation. *Frontiers in Nutrition*, *10*(June). https://doi.org/10.3389/fnut.2023.1173225

Zeb, A., & Ullah, F. (2016). A Simple Spectrophotometric Method for the Determination of Thiobarbituric Acid Reactive Substances in Fried Fast Foods. *Journal of Analytical Methods in Chemistry*, *2016*. https://doi.org/10.1155/2016/9412767

Zhang, H., Yuan, Y., Zhu, X., Xu, R., Shen, H., Zhang, Q., & Ge, X. (2022). *Physicochemical Properties , and Volatile Compounds from.*

Zhang, Q. W., Lin, L. G., & Ye, W. C. (2018). Techniques for extraction and isolation of natural products: A comprehensive review. *Chinese Medicine (United Kingdom)*, *13*(1), 1–26. https://doi.org/10.1186/s13020-018-0177-x

Zhang, Z., Wang, F., Wang, X., Liu, X., Hou, Y., & Zhang, Q. (2010). Extraction of the polysaccharides from five algae and their potential antioxidant activity in vitro. *Carbohydrate Polymers*, *82*(1), 118–121. https://doi.org/10.1016/j.carbpol.2010.04.031

Zheng, M., Liu, Y., Zhang, G., Yang, Z., Xu, W., & Chen, Q. (2023). The Applications and Mechanisms of Superoxide Dismutase in Medicine, Food, and Cosmetics. *Antioxidants*, *12*(9). https://doi.org/10.3390/antiox12091675