ASSESSMENT OF THE AMELIORATIVE EFFECT OF *TURBINARIA DECURRENS* AGAINST STREPTOZOTOCIN-INDUCED HYPERGLYCEMIA IN WISTAR RATS.

Thesis Submitted for the Award of the Degree of

DOCTOR OF PHILOSOPHY

IN

CLINICAL BIOCHEMISTRY

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DECLARATION

I, Juhi Kataria bearing Registration No.42000553, hereby declare that the research work embodied in the Ph.D. thesis titled **"Assessment of the ameliorative effect of *Turbinaria decurrens* against Streptozotocin-induced Hyperglycemia in Wistar rats"** submitted to Lovely Professional University, is the original and bonafide work carried out by me under the supervision of Dr. Louis Cojandaraj A, Professor, Department of Medical Laboratory Sciences, School of Allied and Healthcare Sciences, Lovely Professional University Phagwara, Punjab, India.

I further declare that:

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CERTIFICATE

This is to certify that the work reported in the Ph.D. thesis entitled **"Assessment of the ameliorative effect of *Turbinaria decurrens* against Streptozotocin-induced Hyperglycemia in Wistar rats"** submitted in fulfillment of the requirement for the award of degree of **Doctor of Philosophy (Ph.D.) in Clinical Biochemistry** from the Department of Medical Laboratory Sciences, School of Allied and Healthcare Sciences, Lovely Professional University Phagwara, is a research work carried out by **Juhi Kataria, Registration No. 42000553,** is bonafide record of her original work carried out under our supervision and that no part of thesis has been submitted for any other degree, diploma or equivalent course.

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ABBREVIATIONS

%	Percentage
µg/mL	microgram per milliliter
µmoL	micromole
ABTS	2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)
AgNps	Silver Nanoparticles
AMPK	AMP- activated protein kinase
CAT	Catalase
CC50	Cancer cell lines
CCK-8	Cell Counting Kit-8
CHOD/PAP	Cholesterol Oxidase Peroxidase Phenol Aminoantipyrine
CSMCRI	Central Salt and Marine Chemicals Research Institute
CTAB	Cetyl Trimethyl Ammonium Bromide
DM	Diabetes Mellitus
DNA	Deoxyribonucleic Acid
DNP-BSA	2, 4-Dinitrophenyl-Bovine Serum Albumin
DPP-4	Dipeptidyl Peptidase-4
DPPH	1,1-diphenyl-2-picrylhydrazyl
EDTA	Ethylenediamine tetra acetic acid
EEZ	Exclusive Economic Zone
FRAP	Ferric Reducing Antioxidant Power
FT-IR	Fourier-transform infrared
GAE/mL	Gallic Acid Equivalents per milliliter
GIP	Glucose Dependent Insulinotropic Peptide
GLDH	Glutamate dehydrogenase
GLP-1	Glucagon-like Peptide -1
GOD/POD	Glucose Oxidase Peroxidase
GPO/PAP	Glycerolphosphate Oxidase-p-Aminophenazone

GPx	Glutathione Peroxidase
HbA1c	Glycated Hemoglobin
HDL	High Density Lipoproteins
HIV	Human Immunodeficiency Virus
HOMA-IR	Homeostatic Model Assessment of Insulin Resistance
HOMA-β	Homeostatic Model Assessment of β cell functioning
IC_{50}	Half Maximal Inhibitory Concentration
IU/L	International Units per Liter
kDA	kilodalton
LDL	Low Density Lipoproteins
LPO	Lipid Peroxidation
LPO	Lipid peroxidation
MEOH	Methanolic Extract
mg/dL	milligrams per deciliter
MIC	Minimum Inhibitory Concentration
MODY	Maturity Onset Diabetes of the Young
MPTP	1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MTT	(3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H tetrazolium)
NADH	Nicotinamide adenine dinucleotide + hydrogen
NDM	Neonatal Diabetes Mellitus
PCR	Polymerase chain reaction
PPAR gamma	Peroxisome Proliferator Activated Receptor Gamma
PPARγ	Peroxisome Proliferator Activated Receptor Gamma
ROS	Reactive Oxygen Species
RP-HPLC	Reverse Phase High Performance Liquid Chromatography
RPM	Revolutions per minute
SD	Standard deviation
SGLT2	Sodium-glucose transport protein 2 (SGLT2) inhibitors

SGOT	Serum Glutamic Oxaloacetic Transaminase
SGPT	Serum Glutamic Pyruvic Transaminase
SOD	Superoxide Dismutase
STZ	Streptozotocin
TBARS	Thiobarbituric Acid Reactive Substances
TD	*Turbinaria decurrens*
TG	Triglycerides
TLC	Thin Layer Chromatography
Type I DM	Type I Diabetes Mellitus
Type II DM	Type II Diabetes Mellitus
TZD	Thiazolidinediones
UV-visible	Ultra violet visible
VLDL	Very Low-Density Lipoproteins
WHO	World Health Organization

ABSTRACT

Diabetes mellitus is one of the most widespread metabolic disorders characterized by persistent hyperglycemia resulting from impaired insulin secretion, reduced insulin action or a combination of both. This metabolic dysregulation leads to progressive dysfunction of vital organs including the liver, kidney, pancreas and cardiovascular system. The prevalence of diabetes continues to rise globally due to sedentary lifestyle, high calorie diet and genetic dispositioning. Traditional therapeutic drugs for Type II DM primarily include oral hypoglycemic agents such as sulfonylureas, meglitinides, biguanides, thiazolidinediones and α-glucosidase inhibitors.

Though these drugs can reduce the blood glucose levels through various mechanisms, prolonged use of these conventional drugs are often associated with drawbacks such as hypoglycemia, gastrointestinal disturbances, hepatotoxicity, and cardiovascular risks. Therefore, there is a growing need for more safer, cost effective and ecofriendly alternatives. To this effect, natural products have gained significant attention, particularly marine macroalgae or seaweeds, rich in polysaccharides, polyphenols, carotenoids, flavonoids and essential minerals many of which exhibit antioxidant, anti-inflammatory and anti-diabetic activities. Earlier researches have indicated that metabolites from seaweeds have the ability to diminish postprandial hyperglycemia by inhibiting carbohydrate digesting enzymes enhancing insulin sensitivity and reducing oxidative stress. Among these the genus Turbinaria has demonstrated promising pharmacological potential, yet systematic studies remain limited.

The current research was undertaken to investigate the pharmacological significance of *Turbinaria decurrens*, collected from Mandapam coast, Tamil Nadu. The study aimed to analyze the phytochemical composition, in-vitro antioxidant activities, *in-vitro* antidiabetic potential through inhibition of α-amylase and α-glucosidase enzymes. The determination of cytotoxicity profile of the extracts on 3T3 fibroblast cell lines was also conducted. The preliminary determination was followed by *in-vivo* acute toxicity and pharmacodynamic studies in streptozotocin induced diabetic Wistar rats to evaluate safety and therapeutic effectiveness. The sample of *Turbinaria decurrens* was collected from

Gulf of Mannar, coast Rameshwaram (Mandapam) Tamil Nadu, India and taxonomically authenticated using standard morphological and genetic identification techniques. A certificate of identification was procured and genomic DNA was isolated from seaweed. The genomic DNA was amplified and sequenced. The sequences analyzed BLAST program at the NCBI website showed up to 99.3% sequences similarity with existing plant *rbcL* gene sequences, specifically with Genbank accession number DQ448830.1. The samples after identification was further dried, powdered and were subjected to extraction with three different solvents acetone, aqueous and methanol. The phytochemical screening was performed to identify secondary metabolites such as alkaloids, coumarins, glycosides, flavonoids, phenols, terpenoids, saponins and tannins. The seaweed derived compounds were also evaluated quantitatively to ascertain their quantification. Furthermore, the seaweed extracts were evaluated for in-vitro antioxidant activities to check insight into the free radical scavenging ability and overall antioxidant potential of the extracts. Multiple assays including ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid), DPPH (1,1-diphenyl-2-picrilhydrazyl), FRAP (Ferric Reducing Antioxidant Power) and LPO (Lipid Peroxidation) assay were performed. In-vitro antidiabetic activity was evaluated by measuring the inhibitory effects of extracts against α-amylase and α- glucosidase enzymes, which are critical in carbohydrate digestion and glucose absorption. Cytotoxicity evaluation was assessed using 3T3 fibroblast cell lines through (3-(4,5- dimethylthiazole-2-yl)-2, 5-diphenyltetrazolium bromide) Assay (MTT) to check the cell viability at different concentrations of the extracts. After the preliminary investigations, the methanol extract was selected to check the safety profile and effective dose for managing diabetes. The Organization for Economic Cooperation and Development (OECD) guidelines 423 (Annexure 2b) were followed for acute toxicity study and all safety protocols established by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) were kept in mind to ensure the well-being of the animals. The animals were procured from NIPER Mohali, Punjab, India after getting approval from institutional animal ethics committee. The observations included general appearance, body weight measurements, behavioral changes, or treatment related mortality.

In addition to, the pharmacodynamic studies was conducted on animals. The animals were made diabetic by inducing streptozotocin /kg of body weight. Wistar rats were divided into six groups (n=8) according to their body weight. Group 1 served as the normal control while Group 2 represented the diabetic control. Group 3 received the standard antidiabetic drug metformin. Group 4, 5, 6 were administered varying dose of the methanolic extract of Turbinaria decurrens. Parameters such as body weight, fasting blood glucose levels, renal and hepatic function tests, lipid profile, hematological parameters and histopathological observations in vital organs (liver and kidney) were systematically assessed. *Turbinaria* decurrens was identified as brown algae belong to the family *Sargassaceae* and characterized by an upright thallus with radially branched axes having blades of tough texture. Phytochemical analysis of all three extracts confirmed the presence of diverse bioactive metabolites namely coumarins, glycosides, phenols, tannins, terpenoids. The quantitative results presented highest number of active constituents in methanolic extract as compared to acetone and aqueous extracts. Antioxidant assays revealed that methanolic extract demonstrated the strongest free radical scavenging ability in ABTS, DPPH, FRAP and LPO assays. The enzyme inhibition assays of seaweed extract also indicated the potent inhibitory effect on α-amylase and α-glucosidase, with methanolic extract showing the highest inhibitory effect among all three extracts. Cytotoxicity analysis showed that all extracts maintained high cell viability exceeding 80% at 200µg/mL confirming a favorable safety profile on 3T3 cell lines.

Furthermore, acute toxicity profile *in-vivo* showed no mortality or behavioral abnormalities across all treatment groups in a 14-day period. General observations of fur condition, appetite and salivation remained normal throughout the study. The hematological and biochemical assessments demonstrated no adverse alterations and histopathological examinations of liver and kidney confirmed the absence of toxicity. Pharmacodynamic analysis revealed that oral administration of methanolic extract of *Turbinaria decurrens* at varying concentrations significantly reduced fasting blood glucose levels in streptozotocin induced diabetic rats in a dose dependent manner.

Kidney function test, liver function test and lipid profiles were improved and were comparable to standard metformin antidiabetic drug. The activities of antioxidant enzymes and lipid peroxidation levels were assessed in tissues of control and experimental groups.

Keywords: *Turbinaria decurrens*, antioxidant, antidiabetic, cytotoxicity, acute toxicity, Wistar rats, streptozotocin.

CHAPTER 1

INTRODUCTION

1.1 Diabetes Mellitus (DM) and its classification: DM is the most prevalent metabolic disorder characterized by chronic hyperglycemia, which leads to the gradual development of various physiological complications. This condition arises either due to the pancreas generating inadequate insulin or the body not effectively utilizing the insulin that is produced (American Diabetes Association, 2021). Numerous environmental and genetic factors are believed to contribute the progression of DM including unhealthy food intake, lifestyle and occupation. The management of diabetes is crucial as untreated or unmanaged diabetes can lead to impairment of vital organs like liver, kidney, pancreas and nerve damage. According to International Diabetes Federation 2024, 589 million adults (aged 20-79years) are living with diabetes which is projected to rise to 853 million by the year 2050 (Genitsaridi *et al*., 2024). According to World Health Organization (2019), DM is categorized into two primary types based on its etiology: insulin dependent diabetes mellitus (Type I DM) and non-insulin dependent diabetes mellitus (Type II DM). The other types included Hybrid form of Diabetes (Immune mediated types of diabetes), Other specific types (monogenic defects of β-cell function), Unclassified diabetes.

Type I DM is mainly caused by immune-mediated destruction of pancreatic beta cells, leading to a complete deficiency of insulin. Type II DM is defined by insulin resistance coupled with a relative defect in insulin secretion. It is most frequently linked to obesity and being overweight, and it generally develops later in life. Other important types included Gestational Diabetes develops during pregnancy, Maturity Onset Diabetes of the Young (MODY) resulting from a single gene mutation and Neonatal Diabetes Mellitus (NDM) that often run in families and appear at a young age.

1.2 Pathophysiology of Diabetes Mellitus: In the context of pathophysiology, the interruption of the feedback loops connecting insulin action and insulin secretion results in unusually high blood glucose levels. Typically, insulin secretion is closely regulated

according to blood glucose levels. Nevertheless, in diabetes whether caused by insulin resistance (Type II) or insulin deficiency (Type I), this feedback mechanism becomes dysfunctional, leading to ongoing hyperglycemia (Kahn *et al*., 2014). In β-cell dysfunction, the pancreas is impaired to produce insulin, thus limiting the body' efficiency in controlling blood glucose levels. Concurrently, insulin resistance promotes an increase in hepatic glucose production and a decrease glucose uptake in muscle, liver and adipose tissue. Though both mechanisms appear early in the course of Type II DM and contribute to the development of the disease, dysfunction of β cells and insulin resistance occur simultaneously leading to exacerbated hyperglycemia which accelerates the progression of Type II DM (Kahn, 2003).

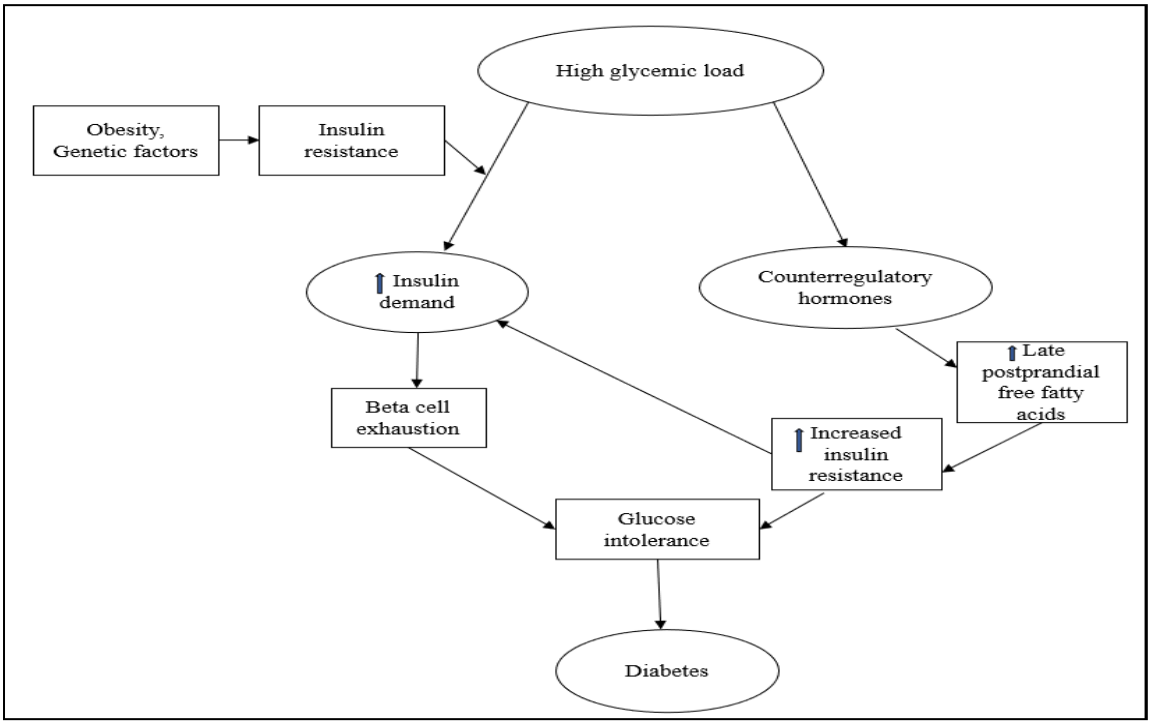


Figure 1: Pathophysiology of Diabetes

1.3 Complexities of Type II DM: DM is a complex disorder involving intricate interactions between genetic predisposition, immune responses, hormonal regulation and environmental influences. Type II DM develops gradually due to a combination of insulin resistance and impaired insulin secretion. Insulin resistance, commonly associated with obesity, physical inactivity, and genetic predisposition, may be present years before clinical diagnosis. Initially pancreatic β-cells compensate by increasing insulin secretion to maintain normal glucose levels. However prolonged metabolic stress eventually leads to β-cells dysfunction and reduced insulin output (Zhao *et al*., 2023). Additionally altered incretin hormone activity, particularly involving GLP-1 and GIP, further impairs glucose stimulated insulin secretion and disrupts glucagon regulation (Zargar *et al*., 2025). Following are the details of complexities of DM.

Insulin resistance: Insulin resistance, largely caused by excess body fat and influenced by genetics, is the main cause of Type II diabetes mellitus. Insulin resistance is estimated to develop 10-15 years before the onset of Type II DM. The development of insulin-resistance typically results in decrease in glucose uptake by the target tissue, most notably in skeletal muscle. Consequently, during an over intake of calories, more insulin is required to allow glucose to move into these tissues. This elevation in insulin, or hyperinsulinemia, further promotes insulin resistance (Freeman *et al*., 2023b). This pathological cycle continues until the pancreatic beta cells can no longer adequately meet the insulin demands creating insulin resistance and resulting in hyperglycemia.

β cell failure: β cells have a vital function to control insulin release in accordance with plasma glucose levels that are kept within a fairly narrow physiological range. In people with diabetes β cells are incapable of releasing enough insulin to meet the increased demand of body (Ferrannini, 2010). As stated above the evolution of Type II DM begins with insulin resistance leads to a rise in β cell insulin secretion in an attempt to oppose the resistance and keep normal blood glucose levels. However, the mass and function of β-cells gradually decrease, resulting in a worsening dysfunctional insulin secretion leading to failure of the β cells to respond (Nolan & Prentki, 2019).

Incretin dysfunction: Gastrointestinal peptides GLP-1 (glucagon-like peptide -1) and GIP (glucose dependent insulinotropic peptide) hormones are released on consumption of food, which also leads to the release of insulin and amylin from β cells of the pancreas in normal persons. GLP-1 is produced from the proglucagon gene in the L cells of the small intestine. GLP-1 and amylin have an inhibitory action on gastric emptying, glucagon release, and on appetite control. After absorption of food, incretin hormones stimulate insulin release; yet in diabetic patients, there is failure of aforementioned processes (Nauck & Müller, 2023).

1.4 Managing diabetes with oral antidiabetic drugs: Currently, there are ten classes of orally active pharmacologic agents intended for the management of Type II DM.

- **Sulfonylureas:** Sulfonylureas introduced in 1950s, are among the first oral medications used to help manage type II DM. The first-generation drugs, like tolbutamide and chlorpropamide, have a low affinity for their receptors, therefore patients often need to take higher doses to achieve therapeutic effects. Because of their limited effectiveness and higher risk of side effects, these older medications have largely been phased out. In contrast second generation sulfonylureas-such as glyburide, glipizide and glimepiride were developed and demonstrated significantly greater potency. These agents are still commonly prescribed as they effectively boost insulin secretion from the pancreas (Thulé & Umpierrez, 2014).

- **Meglitinides:** Meglitinides, including repaglinide and nateglinide belong to the category of non-sulfonylurea pancreas stimulating drugs with a rapid onset and short duration of action. Repaglinide (manufactured in 1998) and Nateglinide (manufactured in 2001) stimulate insulin release in a glucose dependent manner, minimizing the occurrence of hypoglycemia. With a very short half-life of around one hour, these drugs absorbed rapidly. These drugs are most effective in controlling postprandial glucose level. Side effects include hypoglycemia and weight gain (Tahrani *et al*., 2016).

- **Metformin:** Metformin (guanidine analog) has become the most frequently prescribed oral medication for Type II DM because of its efficacy as monotherapy and in combination with other drugs. It gained widespread acceptance after it was discovered that the risk of

lactic acidosis is negligible in patients with normal renal function. Metformin reduces hepatic glucose output and enhances hepatic insulin sensitivity. Side effects may include diarrhea, nausea and abdominal discomfort (Rena *et al*., 2017).

• **Thiazolidinediones:** Troglitazone, pioglitazone and rosiglitazone are thiazolidinediones (TZDs), a group of insulin-sensitizing drugs that stimulate PPAR gamma regulating blood sugar. They decrease insulin resistance, enhancing the body's use of natural and injected insulin. These drugs also improve and preserve β cell functioning (Yki-Järvinen, 2003). Troglitazone was taken off the market in year 2000 because of rare but serious liver toxicity, an adverse effect not associated with pioglitazone or rosiglitazone. Complications may include weight gain in uncontrolled diabetes, congestive heart failure, risk of fractures and edema.

• **Alpha-glucosidase inhibitors:** Acarbose, miglitol and voglibose are α-glucosidase inhibitors oral antidiabetic drugs introduced during the 1990s. These medications work by slowing down carbohydrate digestion within the intestine, helping to reduce post-meal blood sugar spikes in people with Type II DM. These inhibitors are reversible, competitive inhibitors of the enzymes work by inhibiting the breakdown and absorption of carbohydrates thus helping lowers blood sugar levels after meal (Laar *et al*., 2009). Complexities may include gastrointestinal disturbances like abdominal discomfort and diarrhea.

• **Dipeptidyl Peptidase -4 (DPP-4) inhibitors:** Sitagliptin, saxagliptin, linagliptin, alogliptin, and vidigliptin are DPP-4 inhibitors that are very similar to each other with minor differences. It works by preventing the breakdown of the hormones GLP-1 and GIP, thus increasing their levels and activity (Dogruel & Balci, 2019). GLP-1, secreted by intestinal cells stimulate the release of insulin and suppresses glucagon secretion in a glucose dependent manner. GIP stimulate the release of insulin and suppresses glucagon secretion in a glucose -dependent manner. This drug has minimal and rare side effects. Sometimes very rare hypersensitivity reactions may occur.

- **Bile acid sequestrants:** Colesevelam is a non-absorbed bile acid-binding drug developed to lower LDL cholesterol. It was later found to improve blood glucose control and is approved for treatment of Type II DM. Other bile acid sequestrants, including cholestyramine, may have similar effects on lowering glucose levels (Hansen *et al*., 2014). The exact mechanism by which bile acid sequestrants improve glucose metabolism is unclear and controversial. Adverse effects may include gastrointestinal distress viz constipation, complain of bloating and aggravation of hemorrhoids.

- **Dopamine agonists:** Bromocriptine, a quick release agonist of dopamine D2 receptor, was approved to improve blood sugar control in patients with Type II DM. Originally it was used to treat hyperprolactinemia and Parkinson's disease. Bromocriptine is a central-acting drug that can be used either as monotherapy or with other diabetes medications (Lamos *et al*., 2016). It is also used to decrease post meal plasma free fatty acids and triglycerides levels. Difficulties with this drug may include nausea, vomiting, dizziness and seizures.

- **Sodium-glucose transport protein 2 (SGLT2) inhibitors:** Canagliflozin, Empagliflozin, Dapagliflozin and Ertugliflozin are SGLT2 inhibitors that demonstrated efficacy in reduction of HbA_1c. SGLT2 is found in the kidneys and has an important function in reabsorbing most of filtered glucose in the proximal tubules. They are effective in lowering glucose levels and it worked well when blood glucose levels are less than 180mg/dl; however, high glucose levels cause increased excretion of glucose in urine. There are more SGLT2 transporters in patients with Type II DM than normal, which results in increased reabsorption of glucose and blood glucose levels close to 220mg/dl before glycosuria develops. SGLT2 inhibitors cause excretion through the urine leading to loss of 60-90 grams of glucose daily (Thomas & Cherney, 2018). Diminished renal function leads to a decrease in filtered glucose and less excretion through the urine, while high blood glucose resulted in increased filtered glucose and increases the loss of glucose in the urine. Adverse reactions included genital mycotic infections, urinary tract infections and fractures etc.

• **Oral glucagon-like peptide 1 (GLP-1) receptor agonists:** Oral semaglutide a 31 amino acid peptide, is more effective in lowering A1c than either DPP-4 or SGLT2 inhibitors, and equals liraglutide in effect, although less potent than injectable semaglutide. This drug includes a permeation enhancer, N-(8-[2-hydroxybenzoyl] amino caprylic acid, which aids absorption across the stomach lining by protecting the peptide from enzymatic hydrolysis and acid breakdown, thus allowing effective uptake of the whole drug (Hedrington & Davis, 2019). The most common side effects include constipation, diarrhea, vomiting and nausea. The risk of severe hypoglycemia is less common.

Diabetes care and management remains challenged by progressive β cell decline and limited long-term metabolic repair even with major advancements in pharmacotherapy. Current therapeutic strategies focus on specific pathways such as insulin secretion, insulin sensitivity or incretin enhancement. But given that diabetes is a multifactorial condition, it's possible that single target therapies won't adequately address the complexity of the condition. Although existing pharmacological classes offer mechanism based targeted therapies, they also linked with limitations such as risk of hypoglycemia, weight gain and cardiovascular disorders. Consequently, there is a growing scientific interest in multi target bioactive compounds capable of modulating oxidative stress and carbohydrate metabolism simultaneously. This provides a rationale for investigating marine derived phytochemicals as supplemental metabolic modulators.

1.5 Seaweeds, importance and need for alternative herbal medicine for the treatment of Type II DM: Owing to the multifactorial nature of the pathogenesis of diabetes and limitations of the present therapeutic strategies there is a compelling need to search for bioactive compounds that could have effects on antioxidant and glycemic regulatory effects. Seaweeds also known as marine macroalgae, have surfaced as potential options in the field of alternative medicines for controlling diabetes. These macroalgae are rich in bioactive compounds, including polysaccharides like fucoidan, alginate and laminarin; polyphenols, carotenoids and essential minerals which exhibit different health benefits (Lomartire *et al*., 2021). Conventional therapies, including oral hypoglycemic drugs and

insulin therapy, commonly carry side effects such as gastrointestinal upset, gain in weight, and susceptibility to risk of hypoglycemia viz seizures, nausea and vomiting. These limitations have increased interest in alternative and complementary therapies, particularly those derived from natural products (Newman & Cragg, 2020). Many of the compounds present in seaweeds exhibited anti-diabetic activities including inhibition of enzymes involved in carbohydrate digestion leads to reduction of the post prandial spikes in blood glucose levels. It can also enhance insulin sensitivity and glucose cell-permeability facilitation. Sulfated polysaccharides like fucoidan have immunomodulatory activity, they may help control inflammatory cytokines that are implicated in autoimmune reactions thereby contributing to β-cells protection (Yu *et al*., 2017). The compounds in seaweeds also have anti-inflammatory and antioxidant effects that help in reducing oxidative stress and inflammation caused by diabetes (Cornish *et al*., 2015). While the biological activity of compounds derived from seaweeds appears promising, their use in therapy needs to be assessed in terms of pharmacokinetics, dose standardization and safety testing before they can be considered for clinical use. The inclusion of seaweed extracts in dietary or therapeutic regimens can constitute a complete and side effect free approach to treating Type II DM specifically in region where access to modern medical treatment is limited. Given the widespread occurrence of diabetes across the world, especially in low- and middle-income countries, the need for safe and cost-effective treatment has become even more urgent than before. In this context, seaweeds particularly brown algae have garnered significant attention owing to their rich content of bioactive compounds (Remya, *et al*., 2022). *Turbinaria* species, are of particular interest since they have demonstrated considerable bioactivities in a number of studies. Their mode of action relates to their high content of fucoidans, phlorotannin, polyphenols, flavonoids and dietary fibers. The pharmacological potential of isolated compounds from *Turbinaria* species is particularly noteworthy, with reported bioactivities that include antiproliferative (anticancer), antipyretic (fever suppressant), anti-inflammatory, antidiabetic, anti-obesity, antiviral, antimicrobial, cardioprotective, hepatoprotective and hypolipidemic (lipid-lowering)

activities (Rushdi *et al*., 2021a). The wide range of biological activities reported are associated with diverse range of bioactive compounds naturally occurring within algae. These comprise of sterols known for their cholesterol-lowering effect and anti-inflammatory activity, amino acids possessing antioxidant activities, fatty acids beneficial against cardioprotective functions, alcohols possessing antimicrobial and cytotoxic activities. Together these compounds add to the varied health advantages of *Turbinaria* making it an important source of new natural products for pharmaceutical, nutraceutical and functional food applications.

1.5.1 *Turbinaria decurrens* Bory de Saint-Vincent taxonomical classification: commonly known as the triangular sea bell, is a brown alga that is found in the family *Sargassaceae*. The taxonomical classification of *Turbinaria decurrens* include; kingdom *Chromista*, phylum *Ochrophyta*, genus *Turbinaria* and species *decurrens* (Guiry & Guiry, 2020). It is commonly found in tropical and subtropical shallow marine habitats, especially in places such as the Indian and Pacific Oceans. The species are widely distributed across the Indo-pacific region including coastal regions of India, Sri Lanka, Southeast Asia, Australia and parts of the western Pacific Islands (Phang *et al*., 2016). It typically found in shallow waters, especially on reef flats, rocky shores and sometimes in deeper lagoons. The name "triangular sea bell" was coined due to the distinctive triangular shape of the thallus of the algae which gives it a bell-like appearance, particularly when it floats on the water surface. It is also characterized by a thick rigid stem-like structure; stipe. The leaf like blades is decurrent, meaning they extend downward along the stipe (Wynne, 2002). *Turbinaria decurrens* plays several vital roles in marine ecosystems as its dense structure provides shelter and substratum for a variety of invertebrates. By reducing wave energy and stabilizing sediments, it helps in the protection of coral reef ecosystems and coastlines. Research has showed that *Turbinaria decurrens* contains a wide range of bioactive compounds including phlorotannin, terpenoids, flavonoids, fucoidans and proteins.

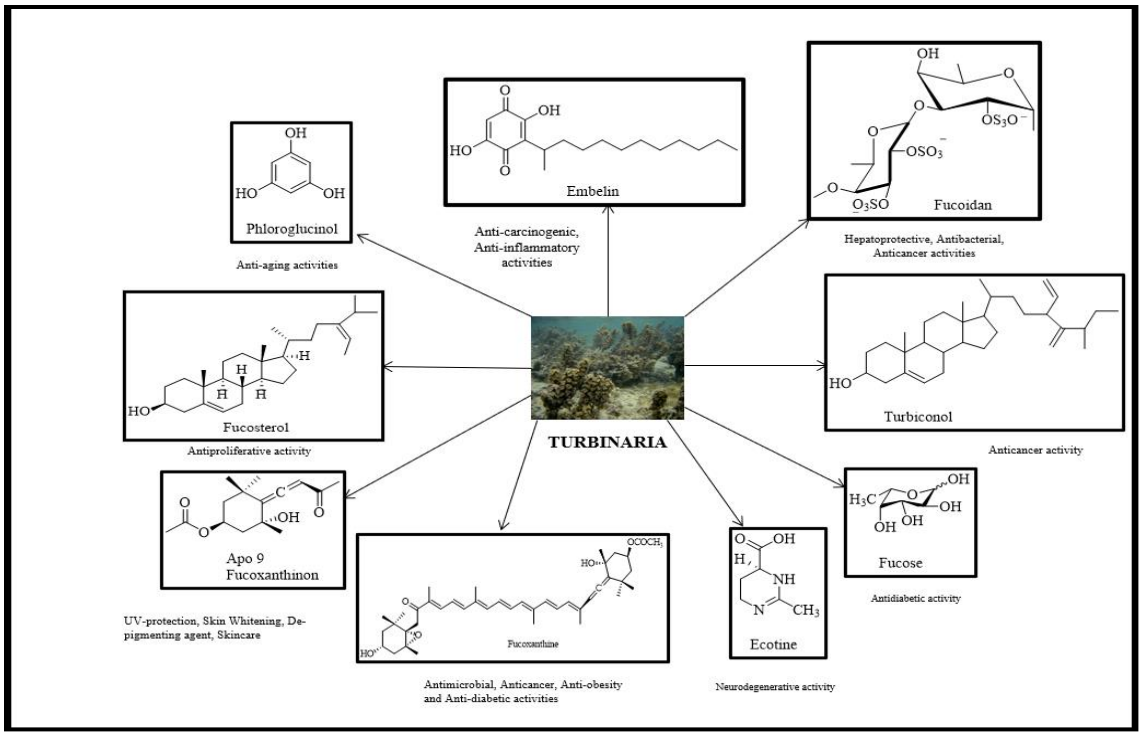


Figure 2: Secondary metabolites of *Turbinaria*

1.5.1.1. Phlorotannin: These compounds (belong to a unique group of polyphenols primarily found in brown algae) are oligomers or polymers derived from phloroglucinol units. Extensive research has shown that these metabolites possess remarkable antioxidant, antimicrobial, anti-inflammatory and anticancer properties. According to recent studies (Kalimuthu & Shankar, 2021) the molecular weight and total phlorotannin content in *Turbinaria decurrens*, was identified by using UV-visible spectroscopy and RP-HPLC (Reverse Phase High Performance Liquid Chromatography). The investigations highlighted notable antioxidant and antibacterial properties of *Turbinaria decurrens.* Acidified methanolic extract exhibited significant free radical scavenging activity as evidenced by DPPH (1,1-diphenyl-2-picrylhydrazyl) and ABTS (2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) assays with IC_{50} values ranging from 49-70µg GAE/mL. This strong reducing potential is primarily attributed to the high content of phlorotannin present in the extract (Arguelles *et al.*, 2020). Furthermore, purified phlorotannin fraction demonstrated marked antibacterial, anti-inflammatory, and

antioxidant effects particularly against methicillin-resistant *Staphylococcus aureus* with minimum inhibitory concentration (MIC) of 125µg/mL underscoring the therapeutic relevance of phlorotannin isolated from *Turbinaria decurrens*.

1.5.1.2. Terpenoids: Terpenoids exhibited a diverse array of anti-tumor activities, are among the most diverse classes of naturally occurring compounds and biologically significant members of this class. According to researchers (Sami *et al*., 2019) the observed DPPH radical scavenging in *Turbinaria decurrens* is the predominance of secondary metabolites such as steroids and terpenoids in its chemical composition. The relatively high abundance of these bioactive terpenoid and steroidal constituents enhances their antioxidant potential, in turn influencing the overall DPPH inhibition efficiency measured in the extract.

1.5.1.3. Flavonoids: Flavonoids are among the key bioactive constituents identified in *Turbinaria decurrens*. Among this quercetin has been reported the most prominent flavonoid extracted, isolated from ethyl acetate fraction and structurally characterized. Despite its moderate levels, it has been recognized as a major contributor to the seaweed's antioxidant and anticancer activities. In a study documented by Sami *et al*., 2021, *Turbinaria decurrens* demonstrated potent free radical scavenging ability with a DPPH IC_{50} 4.23µg/mL and exhibited significant cytotoxicity against lung cancer cells with an IC_{50} of 10.95 µg/mL highlighting therapeutic potential of its flavonoid content.

1.5.1.4. Fucoidan: It is a sulfated polysaccharide from *Turbinaria decurrens***,** having significance in bioactivity and nanotechnology. In a study proceeded by Shanthi *et al*., 2021, fucoidan from the dried seaweed was obtained with crude and purified yields of 5.58% and 1.28% respectively which showed anticoagulant activity, which makes it a potential natural alternative for synthetic blood thinners. Fucoidan was employed in synthesis of silver nanoparticles (AgNps), where it played a role as both a reducing and stabilizing agent. It also showed excellent antibacterial activity against Gram negative bacteria.

1.5.1.5. Proteins and peptides: *Turbinaria decurrens*, is recognized for its diverse range of proteins and polypeptides that play crucial roles in structure, metabolism and bioactivities. The studies conducted so far highlighted its promising applications in nutraceutical and pharmaceutical fields. The protein content in *Turbinaria decurrens* is roughly 5-15% of its dry weight, varying with environmental conditions. The photosynthetic proteins like fucoxanthin-chlorophyll a/c binding proteins, along with metabolic enzymes such as polyphenol oxidase. Preliminary research indicated that the bioactive peptides from this species exhibited antioxidant, antimicrobial and anticancer effects. Moreover, peptides from *Turbinaria ornata* have also shown cytotoxic properties against cancer cells (Remya, *et al*., 2022).

CHAPTER 2

REVIEW OF LITERATURE

2.1 Marine natural products in diabetes management: Diabetes mellitus is a long-term metabolic condition marked by high blood glucose and reduced insulin function (American Diabetes Association, 2021). Although conventional treatments are available, they often come with unwanted side effects and may not provide lasting solutions. As a result, natural compounds from marine macroalgae have become a focus of interest for developing safer and more effective alternatives. Brown seaweeds, particularly those from *Turbinaria* genus, are known to produce a variety of beneficial compounds such as fucoidan, phlorotannin, polysaccharides and fucoxanthin (Rushdi *et al*., 2021b). These bioactive compounds have evolved in marine ecosystems to serve essential protective roles for algae, such as protection against harmful ultraviolet rays and microbial infections. Their chemical structure is often highly specialized making them promising candidate for pharmaceutical research and drug discovery (Rushdi *et al*., 2020). These natural substances have been found to help manage diabetes by blocking enzymes involved in carbohydrate digestion, improving insulin action, and protecting cells from oxidative stress.

2.2 Marine organisms bioactive potential: Marine organisms harbor vast potential as rich reservoirs of highly bioactive secondary metabolites, offering valuable prospects for the development of novel pharmacological agents (Iwamoto *et al*., 2001). Over the previous seven decades, considerable advancement has been achieved in identifying new compounds from marine species, many of which exhibit intriguing biological properties. More than 14,000 distinct products have been identified since the discovery of sponge-derived chemicals, many of which are presently participating in clinical studies to treat ailments such as cancer, pain, or other conditions (Proksch *et al*., 2003). Algae, scientifically referred to as photosynthetic eukaryotic microorganisms, encompass a diverse and ubiquitous group found in various aquatic habitats. They constitute a polyphyletic assemblage, comprising a wide array of taxonomic groups. The varieties of algae encompass both microalgae and macroalgae, representing distinct categories within

the algae taxonomy. Microalgae refer to microscopic unicellular or colonial algae often characterized by their small size, quick growth rates, and excellent photosynthetic efficiency. While, macroalgae encompass larger, multicellular, more complex organisms found in both freshwater and marine habitats typically visible to the naked eye. Macroalgae, commonly known as seaweeds, are anchored to substrates like rocks and shells in ocean depths of up to 180 meters, exhibiting a wide range of sizes, shapes, colors, and textures. Seaweeds play vital ecological roles as primary producers and habitat providers, contributing to nutrient cycling and biodiversity in marine ecosystems. They are abundant in bioactive substances, such as polysaccharides, proteins, lipids, vitamins, minerals, and secondary metabolites, which possess various biological activities such as antioxidant, antimicrobial, and anti-inflammatory properties (Beltagi *et al*., 2022). India's extensive coastline, stretching 8,100 kilometers, supports a variety of coastal ecosystems, including beaches, coral reefs, estuaries, and mangroves (Lakshmi, 2021). This vast coastline is home to wide variety of marine life including a diverse array of marine life. The country's Exclusive Economic Zone (EEZ) spans approximately 2.17 million square kilometers, with about 30% of the global population relying on marine and coastal resources (Ganesan *et al*., 2019). India's diverse coastal ecosystems provide a conducive environment for the growth of economically significant seaweed populations. For instance, the Central Salt and Marine Chemicals Research Institute (CSMCRI) has documented the seaweed diversity along the coasts of Gujarat and Kerala, identifying 366 species, representing approximately half of the variety of seaweeds in India (Jha *et al*., 2022).

2.3 Seaweeds and its classification: Marine seaweeds are renewable resources, increasingly recognized for their various applications, regenerative capabilities, and abundance. They are rich in nutrients, including vitamins, proteins, carbohydrates, minerals (including phosphorus, sulfur, calcium, magnesium, potassium, chlorine, and sodium), and micronutrients (such as iodine, iron, zinc, copper, selenium, molybdenum, fluoride, manganese, boron, nickel, and cobalt) (El-Beltagi *et al*., 2022). Based on their pigmentation, seaweeds are categorized into three taxonomic groups: green algae (Chlorophyta), red algae (Rhodophyta), and brown algae (Ochrophyta). Green algae derive

their coloration from the dominance of chlorophyll a and chlorophyll b pigments, ranging from tiny unicellular forms to large multicellular organisms. Red algae are multicellular (Xu *et al*., 2023), lack centrioles and flagella, with the few unicellular red algae predominantly being extremophiles, thriving in acidic hot springs. These algae vary in color from red to pink to purple (Babich *et al*., 2022), although their growth often shows in multicellular forms in a range of depths from the surface to the deep sea. Brown algae occur in several of the biggest seaweeds, and are mostly of marine habit. The algae have a significant ecological impact and are a major source of food for marine life (Din *et al*., 2022). The brown algae are particularly very nutritious and have medical potential. Their specific features are attributed to their high polysaccharide content, which leads to numerous diverse physical and chemical properties, allowing advantageous interactions with biological systems (Li *et al*., 2021).

2.4 Genus *Turbinaria*, importance & growth Pattern: *Turbinaria* genus was first described by J.V. Lamouroux in 1825. Mainly found within tropical and subtropical marine regions *Turbinaria* usually grows on stony reefs. This genus includes 33 species according to the database, with 22 taxonomically recognized (Guiry & Guiry, 2020). Some of the common species include *Turbinaria capensis*, *Turbinaria conoides*, *Turbinaria conoides*, *Turbinaria costata*, *Turbinaria crateriformis*, *Turbinaria decurrens*, *Turbinaria denudata*, *Turbinaria elatensis*, and *Turbinaria filamentosa*. This genus of brown algae mainly lives on rocky surfaces in tropical marine environments (Blomquist, 1945), but also found in temperate and subtropical regions, besides living on tropical coral reefs. The greatest diversity of the genus is observed in Southeast Asia and the Indian Ocean. According to Zubia *et al*., 2020 *Turbinaria* can survive in a wide range of different settings, including rocky intertidal zones, forereefs, and tide pools. It can grow at depths of about 30 meters and is tolerant to a variety of exposure. The genus is iron rich possesses a preference for nickel and arsenic, depending upon the concentration within the environment. The carotenoid pigment contained by *Turbinaria* is fucoxanthin. Thus, in all the brown algae, this pigment participates in energy transfer and light harvesting (Lopes *et al*., 2021). The pattern of growth of *Turbinaria* consists of side branches emerging laterally from the

central axis of the thallus, which gives rise to a branching structure from one central axis. The leaves bear various morphologies viz shield-shaped (peltate), top-shaped (turbinate), other hard structures, usually with toothed or serrated edges (Lourenço-Lopes *et al*., 2021). Depending on the species, the outer surfaces of the leaves may either be flat, concave, triangular, or rectangular. The variation in the morphology of leaf distinguishes different species of *Turbinaria*. Various species are identified by complex leaf forms, branching patterns, and adaptations that help them to survive in marine environments (Wynne, 2002). In this alga, the individual plant body thallus is connected by an intricate lattice of spreading branches arising from the main axis, giving the overall stability and structure to the algae. The receptacles are located on the upper sides of the leaf stalks. These structures play a very significant role in the algae's reproductive cycle. In fact, many species of *Turbinaria* contain air vesicles within the peltate portion of the leaves. This imparts the thallus, tendency to floats closer to the surface of the water and allows sunlight access for photosynthesis.

2.5 Chemical composition of *Turbinaria* species: Mineral composition is an important nutritional attribute of *Turbinaria* species, consistent with observations across other brown algae. Studies reported that essential macronutrients such as calcium, magnesium and potassium are present in significant concentrations in *Turbinaria* highlighting its dietary importance (Wahbeh *et al*., 1985). *Turbinaria* species often show comparatively low sodium: potassium which can help counteract high sodium potassium dietary patterns that contribute to cardiovascular risk (Matanjun *et al*., 2009). Furthermore, trace elements especially iodine and iron are also present in *Turbinaria ornata*, in a modest concentration comparable to other members of fucales (Zubia *et al*., 2003) making it a good source of iron for people with iron deficiency anemia (Mišurcová *et al*., 2011).

2.6 Valuable phytochemical constituents of seaweed: Phytochemical studies on marine algae have consistently demonstrated the presence of bioactive compounds notably coumarins, glycosides, phenolics, tannins and terpenoids each contributing distinct bioactivities. These compounds are not only vital for the survival and ecological adaptation of algae but also contribute significantly to their pharmacological properties.

Phenolic compounds and tannins are among the most widely studied bioactive compounds due to their strong antioxidant potential, help mitigating oxidative stress associated with chronic metabolic disorders. Terpenoids exhibit broad spectrum activities such as anti-inflammatory, antioxidant and enzyme modulating effects that are directly related in glycemic control. Recent studies have highlighted the therapeutic potential of bioactive compounds from marine sources especially lowering the risk of chronic metabolic disorders like diabetes and cardiovascular diseases. The study also highlighted the importance of refining extraction methods to enhance the yield and effectiveness of these compounds (Kumar *et al*., 2023). The vital role of terpenoids derived from seaweeds was demonstrated by (Chen *et al*., 2024) detailing the anticancer properties, molecular mechanisms and signaling pathways involved in tumor growth. These studies emphasize on preventive and therapeutic role of marine derived natural products in healthcare. Furthermore, coumarins are well known for their anti-inflammatory activities due to variation in its benzopyran structure. The anti-inflammatory activity is directly linked to an aromatic group to an amide bond (Arora *et al*., 2014). Phenolic compounds including phlorotannin are prominent constituents of brown algae and exhibit strong free radical scavenging capacity, which helps mitigate oxidative stress implicated in chronic disease such as diabetes (Meng *et al*., 2021). Significant potential of seaweeds as source of unique bioactive compounds highlighting their nutritional, medicinal, antimicrobial was described in another study, emphasizing that seaweeds are not only rich in essential nutrients but also serve as secondary metabolites with pharmacological significance (Rengasamy *et al*., 2020).

2.7 Therapeutic compounds derived from *Turbinaria:* Compounds derived from *Turbinaria* possess potential medicinal activities, such as antiviral, antibacterial, cytotoxic, antipyretic, anti-inflammatory, hypolipidemic, hepatoprotective, antidiabetic and cardioprotective (Rushdi *et al*., 2020). Some of the compounds have exhibited antiviral and antimicrobial activity, showing promising natural alternatives to synthetic antimicrobial agents (Remya, *et al*., 2022). The hepatoprotective properties of some compounds may benefit individuals with liver diseases by helping protect the liver from damage (Yang *et*

al., 2019). For instance, *Turbinaria* fucose constitutes most of the fucoidan, a sulfated polysaccharide, and also contains simple sugars such as galactose, xylose, and glucuronic acid. The potential health benefits of fucoidan, particularly its anticancer properties, have garnered considerable interest among medical scientists (Sheekh *et al*., 2023). Fucoidans extracted from *Turbinaria ornata* using enzyme-assisted extraction also found to display strong levels of in vivo anti-inflammatory activity, as assessed through FTIR spectroscopy and tested on RAW 264.7 macrophage cell lines. The results of the experiment demonstrated that fucoidan efficaciously inhibited inflammation caused by lipopolysaccharides, thus having a potential application for both the pharmaceuticals and the cosmeceuticals industries (Fuentes *et al*., 2014). It also exhibits anticoagulant properties, with the majority of sulfate groups at the C3 and C6 positions of galactose, while fucoidan with anticoagulant activity has been identified at the C4 and, to a lesser extent, C2 positions of the (1–3)-α-l-fucopyranosyl residue. Silver nanoparticles (AgNPs) with potent anticoagulant properties that are produced with isolated fucoidan efficacy and high antimicrobial activity against gram-negative pathogens (Shanthi *et al*., 2021). Among the various pharmacological properties of fucoidans, their antiproliferative efficacy against the lung cancer cell line of humans A549 has been emphasized in studies by Boo *et al*., 2011 and Jin *et a*l., 2022. Researchers have also identified fucoxanthin from *Turbinaria decurrens* as a potential treatment for colon cancer. Cytotoxicity assays using the MTT assay (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H tetrazolium) and Cell Counting Kit-8 (CCK-8) have shown that *Turbinaria decurrens* inhibits proliferation and triggers apoptosis in human colorectal cancer cell lines (Zakaria et al., 2018; Nurrochmad *et al*., 2018). Potential use of fucoxanthin for colorectal cancer treatment has been supported by various research findings (Shimoda et al., 2010). The antibacterial properties of fucoidan extracted from *Turbinaria ornata* extract were examined against a range of pathogenic microorganisms, both Gram-positive and Gram-negative, with polyphenols being identified as the key active agents. These phenolic compounds, based on their composition, significantly influence bacterial growth and metabolism, supporting the use of *Turbinaria ornata* methanol extracts as potent

antimicrobial agents (Vijayabaskar *et al*., 2011). Fucosterol, a sterol found in brown seaweeds is known for their several bioactivities, including anti-inflammatory, anticancer, immunostimulant, hepatoprotective, antioxidant, anti-obesity and antibacterial effects (Shanmugam *et al*., 2010). Several metabolites, which include oxygenated fucosterols, tanacetol A, and embelin, have drawn much interest owing in relation to their unique intriguing pharmacological characteristics and intricate structure. Isolated fucosterol from *Turbinaria conoides* gathered from the Gulf of Mannar, India that showed potent antibacterial activity against *Staphylococcus aureus*, *Staphylococcus epidermidis, Escherichia coli*, *Pseudomonas aeruginosa, Aspergillus niger,* and *Candida albicans*. Sheu *et al*., 1999 showed the cytotoxicity of fucosterol isolated from on several cancer cell lines where structures were justified by spectral analysis. Other sterols, such as 24ξ-hydroperoxy-24-vinylcholesterol, were found in *Turbinaria tricostata* extracts with cytotoxic activity on the cell lines of cancer cell line CC50 ranging from 14.8 to 41.2 µg/mL.

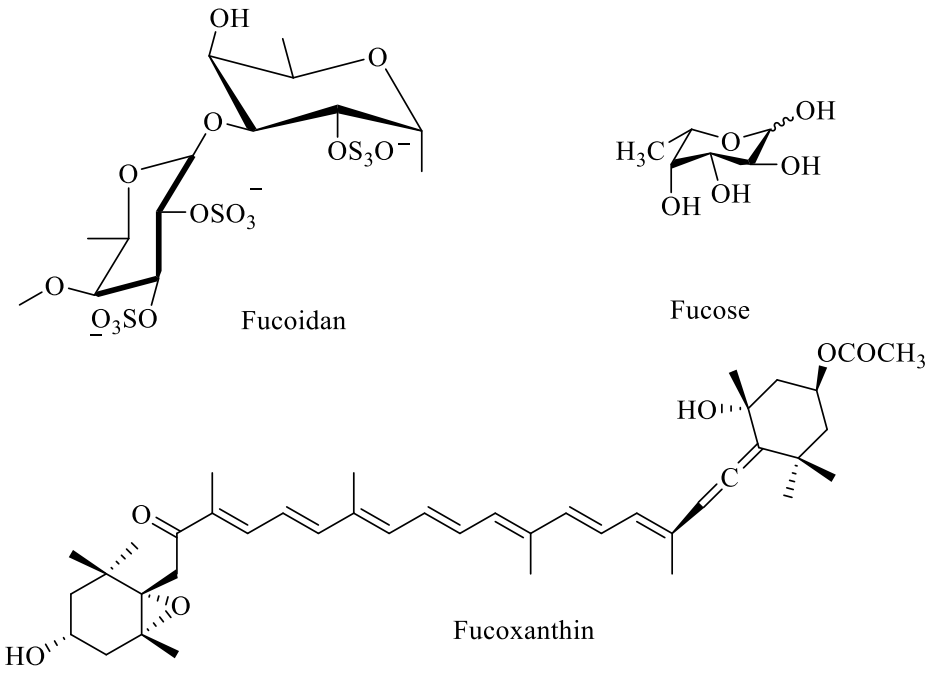


Figure 3: Chemical structures of Fucoidan, Fucose, Fucoxanthin

(Chemdraw ultra 8 software was used to draw the chemical structures)

Phlorotannin, a subset of phenolic compounds, are synthesized in seaweeds through the polymerization of phloroglucinol through the acetate-malonate pathway. In brown algae, phlorotannins, are usually produced under extreme environmental conditions empowering the compounds having the capacity to take in UV radiation and aid wound healing (Jayawardena *et al*., 2019), such polyphenols play a vital role in cosmeceutical and nutraceutical formulations owing to its broad health implications (Cotas *et al*., 2020; Sanjeewa *et al*., 2016). Phlorotannins are hydrophilic compounds that exhibit molecular weights ranging from 126kDa to 650kDa (Shimoda *et al*., 2010) and are known for their diverse biological processes namely photoprotection, radioprotection, anti-allergic, anti-diabetic, antioxidant, antiproliferative, and anti-HIV (Sugiura *et al*., 2021). Studies have demonstrated that natural phlorotannin extracted from *Turbinaria ornata* hinder the expression of chemical namely histamine release from histamine releasing cell line RBL-2H3 mast cells (Ragan & Glombitza, 1986). These phlorotannin have also showed antiallergic activity against DNP-BSA (2, 4-Dinitrophenyl-Bovine Serum Albumin) and compound 48/80-induced anaphylaxis (Sugiura *et al*., 2021). Other phlorotannin isolated from *Turbinaria ornata* exhibit antioxidant properties, modulating the transcriptional levels of specific genes [(SOD1 (superoxide dismutase type 1), GPx (Glutathione Peroxidase), CAT (catalase), NRF2 (nuclear factor erythroid 2–related factor 2), BCL2 (cell leukemia/lymphoma 2 protein), and BAX (Bcl-2-associated protein x)] that mediate reactive oxygen species (ROS) activity in RAW 264.7 cells (Kalimuthu & Shankar, 2021). Phlorotannin also activate the NRF2 signaling pathway in primary mouse splenocytes (Girija *et al*., 2013). In addition to the anticancer, antioxidant, antidiabetic, and antiallergic activities of *Turbinaria ornata*, its metabolites have shown antiviral and antimicrobial properties, indicating the genus's wide-ranging potential for disease management (Shanmugam *et al*., 2010). According to recent studies by Kalimuthu & Shankar, 2021 the molecular weight and total phlorotannin content in *Turbinaria decurrens* was identified by using UV-visible spectroscopy and RP-HPLC (Reverse Phase High Performance Liquid Chromatography). The isolated phlorotannin fractions displayed the extract’s important antibacterial, anti-inflammatory, and antioxidant activities. Another research conducted by

Girija *et al*., 2013 confirmed the antioxidant capacity of phlorotannin present in *Turbinaria ornata* methanolic extract on the basis of Thin Layer Chromatography (TLC), UV-Visible and Fourier-transform infrared (FT-IR) spectroscopy spectral analysis F5 fraction. Vijayabaskar & Shiyamala, 2012 also proved that *Turbinaria ornata* contains high levels of phenolic compounds, confirmed by FT-IR and TLC, and possess strong activity that scavenges free radicals. Moreover, ABTS and DPPH free radical assays of *Turbinaria decurrens* methanolic extract had demonstrated polyphenol content, indicated wide-range antioxidant activity. This extracts also exhibited a pronounced ability to reduce copper ions (Arguelles *et al*., 2020).

2.8 Biological properties of seaweeds:

2.8.1 Antidiabetic Activity: Previous studies into enzymatic inhibition potential have demonstrated by many researchers. For instance, in vitro antidiabetic activity of crude extracts of *Turbinaria ornata* have been evaluated by Unnikrishnan *et al*., 2014 through inhibition of carbohydrate hydrolyzing enzymes. Significant inhibitory activities were demonstrated by methanolic and acetone extracts against α-amylase and α-glucosidase. Arguelles *et al*., 2020 also demonstrated in vitro inhibitory activity of α-glucosidase, highlighting its potential as natural antidiabetic drug superior to the standard metformin and acarbose. Fucoidan is a powerful inhibitor of important digestive enzymes like α-glucosidase and α-amylase. it works as a competitive inhibitor, helps to lower glucose release and absorption in the intestine (Koh *et al*., 2020). Supporting this, (Zhao *et al*., 2018) found that fucoidan extracted from *Undaria pinnatifida* had a significant impact on inhibiting α-glucosidase and α-amylase activities in vitro. Furthermore, researcher discovered that fucoidan from *Fucus vesiculosus* and *Ascophyllum nodosum* showed strong inhibitory effects of both these enzymes suggesting it could serve as a natural therapeutic substance in managing postprandial hyperglycemia (Kim *et al*., 2014). Similarly, Phlorotannins found in brown algae also hinder the activity of α-glucosidase and α-amylase. Extracts from *Sysymbrium linifolium* have shown these inhibitory effects along with antioxidant properties and improved pancreatic β-cell function in diabetic rats (Gheda *et al*., 2023). Supporting these findings (Park *et al*., 2018) reported that phlorotannin rich

extracts from *Ecklonia cava* significantly suppressed α-glucosidase and α-amylase activities, resulting in decreased postprandial blood glucose levels. Furthermore, A study found that extracts from *Turbinaria ornata* effectively inhibited protein glycation in vitro and protected against AGE formation in hyperglycemic *Caenorhabditis elegans* models outperforming standard controls (Gunathilaka *et al*., 2020). Fucoidan derived from *Undaria pinnatifida* has been shown to enhance insulin mediated glucose uptake in adipocytes, reduce lipid accumulation, and downregulate PPARγ (peroxisome proliferator activated receptor gamma) expression. These effects help restore insulin responsiveness without causing adipogenesis (Sim *et al*., 2019). The Structural characteristics of fucoidan, influenced by its sulfate content and molecular size, play a crucial role in its ability to improve insulin sensitivity, lower fasting glucose levels and regulate lipid profiles, partly through AMPK (AMP- activated protein kinase) (Zhao *et al*., 2020). Marine polyphenols including fucoidan and phlorotannin are reported to inhibit NF-κB (nuclear factor kappa light chain enhancer of activated B cells) a transcriptional that is key to inflammation and insulin resistance. This modulation leads to improved insulin sensitivity (Pereira & Valado, 2023).

2.8.2 Anticancer Activity: Cancer continues to be among the most prevalent diseases worldwide, with existing therapies often accompanied by significant side effects. Therefore, developing novel treatments with fewer adverse effects is crucial. Polysaccharides derived from seaweeds present a promising avenue due to their anticancer potential against various types of cancer. These polysaccharides exert their effects through multiple mechanisms, including depolarization of the mitochondrial membrane, DNA damage, arrest in the cell cycle, and nitric oxide production (Bhuyan *et al.*, 2023). Several anticancer properties of fucoidan have been shown, such as the stimulation of apoptosis and the prevention of tumor-induced angiogenesis (Nguyen *et al.*, 2019). It has been shown to be effective against various cancers such as the bladder, breast, colon, liver, prostate, and melanoma. In addition to its potential benefits for gastrointestinal health, fiber from polysaccharides may also help prevent colon cancer. Marudhupandi *et al*., 2015 researched the anti-proliferative activity of fucoidan from *Turbinaria ornata* on cancer

cells and reported a dose-related response. Another researcher documented that hentriacontane compound from *Turbinaria ornata* hexane extract had the highest antiproliferative and antioxidant activity (Deepak *et al*., 2017). Further, antioxidants, including β-carotene, have been shown to be helpful in the prevention of precancerous disorders such as oral leukoplakia (Boopathy *et al*., 2010). Green synthesis of AuNPs via the hydromethanolic extract (HME) of *Turbinaria decurrens* was use to carry out and the antioxidant and anticancer properties of both HME and its AuNPs have been verified as therapeutic nanoparticles to treat oxidative stress-related diseases, like cancer (Hasan *et al*., 2022).

2.8.3 Neuroprotective Activity: A study carried out by (Meenakshi *et al*., 2016) indicated that fucoidan from *Turbinaria decurrens* has neuroprotective effects against MPTP (1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine)-induced rat model of Parkinson's. Myricetin a flavonoid compound in various plants, including *Turbinaria ornata*, also exhibited neuroprotective effects. Rotenone-treated Drosophila melanogaster models of Parkinson's disease are used to evaluate the efficacy of myricetin on neurodegenerative conditions. Myricetin had shown to delay neurodegeneration, maintain dopamine levels, prevent apoptosis and oxidative stress via regulating oxidants and antioxidants balance (Dhanraj *et al*., 2017). An experiment was conducted to check the inhibitory potential of *Acetylcholine (*ACh) and Butyrylcholin (BuCh) esterase enzymes in a fraction of *Turbinaria ornata*. ACh and BuCh are some important neurotransmitters required for the proper performance of brain signal transmission as well as the electrical impulses that go between neurons in the synaptic cleft. The methanol-water fraction from *Turbinaria ornata* (MW*TO*) resulted in the induction enhancement of neuronal differentiation, increased 50% neuronal proliferation, with no harmful activities. MWTO, therefore, can work as an excellent protector drug for healthy neurons while replacing the damaged cells, thereby slowing the progression of neurodegenerative diseases (Lingappa *et al*., 2022).

2.8.4 Tissue Engineering: A naturally occurring polymer called alginate is taken from brown seaweeds like *Turbinaria*, *Macrocystis*, *Sargassum*, *Ecklonia*, *Laminaria*, *Lessonia*,

and *Ascophyllum*. These polymers consist of linear chains of D-mannuronic acid and L-guluronic acid units linked by β (1→4) bonds (Abka-khajouei *et al*., 2022). Alginate's unique physicochemical properties, particularly its ability to form gels in aqueous environments, have garnered increasing attention in the disciplines of biomedicine and pharmaceuticals. Crosslinked hydrogels derived from alginate have been developed for applications in tissue engineering and the delivery of bioactive materials. These scaffolds are crucial for regulating the shape and functionality of engineered tissues, facilitating tissue growth and regeneration. Fucoidan-enriched seaweed extracts have shown potential in treating osteoarthritis by enhancing osteocalcin levels and alkaline phosphatase activity, thereby promoting mineral deposition in bones (Cho *et al*., 2009). Alginate-based biocompatible hydrogels are significantly impactful in addressing various organ, bone, and cartilage disorders and are utilized in stem cell transplantation (Barralet *et al*., 2005).

2.8.5 Other biological properties: Algae contain a significant amount fibrous soluble, which is known for its capacity to lower plasma cholesterol, enhance viscosity, and reduce glycemic response. Additionally, these fibers assist in reducing the risk of cardiovascular illnesses and triglyceride levels (Sirot *et al*., 2012; Etherton *et al*., 2009). A study by Krishnamurthy *et al*., 2012 suggested that the sulfated polysaccharides of *Turbinaria conoides* have membrane stabilizing activities that provide protection against isoproterenol-induced myocardial damage, which could be due to the reduction in lipid peroxidation. It may also revive the interest of using *Turbinaria conoides* fucoidan as a possible inhibitor of myocardial injury. Moreover, the anti-HIV potential of *Turbinaria decurrens* is recently documented well (Sanniyasi *et al*., 2019), wherein RP-HPLC and FT-IR analyses were employed to assess the bioactive potential of the sulfated polysaccharide fucoidan obtained from two types of macroalgae: *Dictyota bartayesiana (DD)* and *Turbinaria decurrens*. In addition to this, antibacterial activity against uropathogens was described using silver nanoparticles extracted from *Turbinaria ornata*. Microbes such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, and *Klebsiella pneumoniae*, were tested using the agar well diffusion method. The silver nanoparticles showed increased antibacterial and antioxidant activity, indicating that

they possibly developed as a brand-new class of antibiotic drugs to treat UTIs (Raj *et al*., 2023). Despite the availability of many studies on the antidiabetic potential of brown seaweed extracts, the results are not entirely consistent. This is due to variations in the efficacy of different species and extraction procedures. One of the major drawbacks of the previous studies is the predominant reliance on in vitro enzyme inhibition assays, which fail to represent physiological conditions, dose response activity and relationship between biological activity and phytochemical content are not standardized. Additionally, there are limited in vivo and histopathological studies performed constraining therapeutic use.

2.9 Research gap and need for the study: While several species of *Turbinaria*, like *Turbinaria ornata* and *Turbinaria conoides* have been studied for their biological activities such as enzyme inhibition, antioxidant properties and anti-inflammatory benefits, there is still a noticeable lack of systematic studies on *Turbinaria decurrens* in diabetic models. Most of the findings are limited to in vitro assays, like enzyme inhibition and free radical scavenging activity. There is a lack of animal model studies that affects glucose regulation and overall metabolic health. Additionally, the acute toxicity in vivo and cytotoxicity effects are not studied previously, that helps to determine the safe dose of extracts and how they can be used in the treatment. Comprehensive antidiabetic, antioxidant, cytotoxic and toxicity profiles will provide a better understanding of its therapeutic potential, helping to bridge the existing gap in the literature. This study would not only help confirm its pharmacological value but also enhance the knowledge paving the way for its development as a safe and effective natural remedy.

AIM & OBJECTIVES OF THE STUDY

AIM: To assess the ameliorative effect of *Turbinaria decurrens* against Streptozotocin-induced Hyperglycemia in Wistar rats.

OBJECTIVE:

1) To Collect, Identify, and Prepare crude extract of *Turbinaria decurrens*.

2) To perform phytochemical analysis and antioxidant activity of the crude extract.

3) To identify the ameliorative effect of *Turbinaria decurrens* against streptozotocin induced diabetes in Wistar rats.

4) To find out the potential effect of *Turbinaria decurrens* on the biochemical and histological parameters of hyperglycaemic Wistar rats.

CHAPTER 3

MATERIAL AND METHODS

3.1 SAMPLE COLLECTION AND PROCESSING:

3.1.1 Site of collection: Three different sites at a distance of 50 meter each were chosen to collect the seaweed *Turbinaria decurrens.* The samples were collected from Gulf of Mannar coast Rameshwaram (Mandapam), Tamil Nadu, India (DMS Lat: 11° 7' 37.6428" North DMS Long: 78° 39' 24.8076" East) by handpicked method.

3.1.2 Collection and processing: The seaweed samples were handpicked from the site of collection, and then underwent multiple washing with distilled water to remove sand, debris and other associated organisms such as planktons and microorganisms. The sample were kept in a polythene bag kept under ice and transported to the laboratory. Upon arrival at the laboratory, the seaweed samples were shade dried for a period of eight days before being finely ground using a mechanical blender (Quirós *et al*., 2021).

Figure 4: Map Gulf of Mannar coast Rameshwaram (Mandapam), Tamil Nadu, India

Figure 5: Dried seaweed sample *Turbinaria decurrens*

3.2 IDENTIFICATION OF *TURBINARIA DECURRENS*: The process of identifying *Turbinaria decurrens* involved both taxonomical and molecular approaches, ensuring comprehensive validation of the species.

3.2.1 TAXONOMICAL AND MORPHOLOGICAL IDENTIFICATION: In order to assist with taxonomical classification, herbarium specimen was prepared following the guidelines established by International Centre for Genetic Engineering and Biotechnology (ICGEB), "Seaweed Herbarium," *Department of Biotechnology Apex Biotechnology Information Center*. The specimen was applied on the herbarium sheet using a brush. Once the specimen was mounted, the sheet was gently raised and tilted to one side to allow drainage of excess water without disturbing the mounted specimen. Blotting paper was utilized to absorb any excess moisture. After the specimen was dried, the live and herbarium sample was sent to Sri Herbasia Biotech Pvt. Ltd. Amritsar Punjab, India for its identification. The herbarium was meticulously labelled with the name of the specimen (ICGEB, 2025).

3.2.2 MOLECULAR IDENTIFICATION USING RBCL GENE SEQUENCING:

a) DNA Isolation: The genomic DNA extraction was performed following a specific protocol. The seaweed sample was ground in the presence of liquid nitrogen using a mortar and pestle until it was successively crushed. Genomic DNA was isolated via the Cetyl Trimethyl Ammonium Bromide (CTAB) method outlined by Doyle and Doyle (Doyle_plantDNAextractCTAB_1987,). The lysis solution contained of 100mM Tris (pH 8.0), 20mM EDTA, 1.4M NaCl, 2% CTAB (w/v), and 10mM β-mercaptoethanol. The material was homogenized and then centrifuged at 12000 rpm for 10 minutes to eliminate cellular debris. Following this the mixture was vortexed and incubated at 60°C for 30 minutes. Furthermore, it was treated with an equal amount of chloroform: isoamyl alcohol (24:1). The supernatant was collected after centrifugation at 10000 rpm for 15 minutes at room temperature, later transferred to a sterile centrifuge tube, and treated with 1/10 volume of 3M sodium acetate (pH 5.2) and 1/2 volume of 5M NaCl. The precipitation of DNA took place using 0.6mL cold isopropanol and subsequently pelleted through centrifugation at 10000rpm for 10minutes at a temperature of 4°C. The supernatant was discarded and DNA pellet was washed twice with 70% ethanol. After air drying, the DNA was dissolved in 500µl of 1X Tris-HCl EDTA buffer (TE). RNA contaminants were removed by mixing, Bovine pancreatic RNAase (5mg/mL) to the DNA solution and incubated at 37°C for one hour. A further extraction was performed using chloroform:isomyl alcohol (24:1), and the aqueous phase was transferred to a new tube, where sodium acetate 3M and chilled isopropanol were added for DNA precipitation at -20°C for one hour. The DNA pellet was air-dried and then reconstituted in 50µL of sterile water (Sambrook, 2001).

DNA quantification was conducted spectrophotometrically with a UV-Vis spectrophotometer. 5µL of DNA sample was diluted in 3000µL of slightly alkaline buffer (10mM Tris HCl pH 7.5) and taken in a cuvette. Absorbance measurements were taken at 260nm and 280nm. The concentration was calculated using the following formula.

$$\text{DNA Concentration } (\mu g/mL) = 50 \times OD_{260} / 1000$$

b) Polymerase Chain Reaction (PCR): A PCR-based method was utilized to detect the presence of the *rbcL* gene. Genomic DNA was isolated from the biological sample following the standard extraction protocol. The PCR setup employed specific primers targeting conserved regions adjacent to the *rbcL* gene, along with a high-fidelity DNA polymerase (Mshiywa *et al*., 2024).

The PCR cycling conditions were refined to optimize the amplification of the *rbcL* gene, which included steps for denaturation, annealing and extension. To verify the presence and size of the amplified *rbcL* gene fragment, PCR products obtained were subjected to gel electrophoresis and exposed to UV light. To improve the precision in identifying the target gene, the PCR product was purified and subsequently sequenced. The sequencing of the amplified product was conducted using the ABI PRISM 3730 Genetic Analyzer from Applied Biosystems. The sequences of the *rbcL* primers utilized were as follows:

*rbcL*a- F: (5'-ATGTCACCACAAACAGAGACTAAAGC-3')

*rbcL*a-R: (5′-GTAAAATCAAGTCCACCRCG-3')

The steps of PCR were as followed:

1. DNA denaturation: the double stranded DNA was heated at a temperature of 94°-98°C to separate it into two single stranded.
2. Annealing: the temperature was lowered to 50°-65°C to allow the primers to bind to their complementary sequences on the single stranded DNA templates.
3. Elongation: DNA polymerase synthesized new DNA strands from the primers by adding deoxynucleotide triphosphate in the 5’ to 3’ direction which increased the number of DNA replication.
4. PCR reaction: The reaction was conducted in a 20µL solution comprising 10µL of Takara mix, 1µL each of 10µM forward and reverse primers, and 100-200ng of template DNA. The PCR conditions were as follows: 1 cycle (95°C for 3 minutes),40 cycles (94°C for 45 minutes, 55°C for 45 minutes, and 72°C for 1 minute), and 1 cycle 72°C for 7 minutes (Shah, 2019).

5. Electrophoresis and Amplification: 5µL sample was combined with one-sixth of its volume of 6X loading buffer and subsequently examined using an agarose gel. The gel was then stained with Ethidium Bromide dye to enable visualization under ultraviolet light.

c) Phylogenetic Analysis: The sequences of the *rbcL* gene were compared against the sequences available from GenBank using the BLASTIN program (Zhang *et al*., 2000) and were aligned using CLUSTALW software (Thompson *et al.*, 1994). Phylogenetic tree was constructed using the Maximum Parsimony method (Nei Masatoshi & Kumar Sudhir, 2000). The MEGA11 package (Kumar *et al*., 2018) was used for analysis.

3.3 DRY & WET YIELD OF *TURBINARIA DECURRENS*: To assess the yield of *Turbinaria decurrens* both wet and dry weights of the plant samples were carefully measured. A high precision analytical balance was employed to ensure accurate quantification of material before and after the Soxhlet extraction process. The extraction yield was calculated using a formula (Zhang *et al*., 2022).

Yield (%) = (Weight of Residue/ Initial Sample Weight) × 100

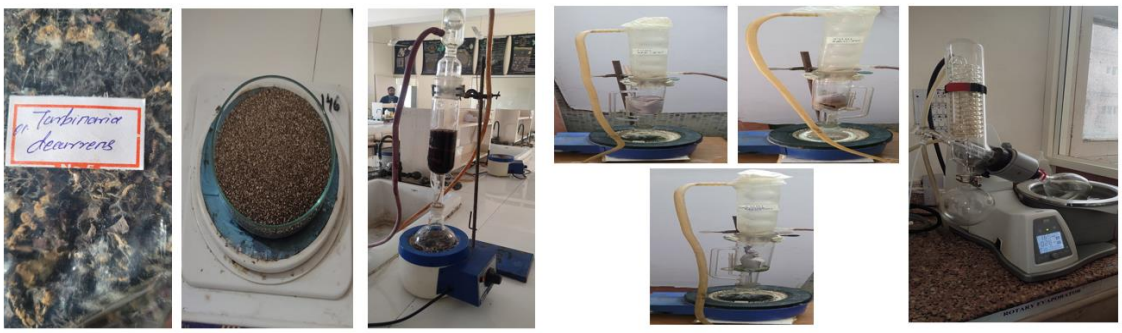
3.4 EXTRACTION OF BIOACTIVE METABOLITES FROM *TURBINARIA DECURRENS:* Hot percolation technique using Soxhlet extraction assembly was employed for the extraction of bioactive metabolites. The acetone, distilled water and methanol were used as solvents. The solvents were applied sequentially from non-polar to polar to ensure comprehensive extraction. The process of extraction included the following steps:

1. 25grams of dried and grinded seaweed was weighed and were kept in a thimble made of filter paper.
2. The components of Soxhlet extraction assembly *viz* Soxhlet extractor, round bottom flask, and a condenser were placed in position on a heating mantle.
3. The selected solvent was heated, vaporized and pass through the condenser where it condensed and dripped into the Soxhlet chamber containing the thimble. Once the

solvent level reached the top of the siphon arm, it automatically siphoned back into the round bottom flask facilitating extraction process. Ten reflux cycles were conducted to ensure thorough extraction of the bioactive compounds.

4. A rotary evaporator (DLAB RE100-Pro Digital Rotary Evaporator) was then used to dry and concentrate the solvents containing bioactive metabolites. The temperature of rotary evaporator was set at 56°C for acetone, 65°C for methanol and 100°C for aqueous solvent.

5. The resulting extracts were then lyophilized to get powdered form extracts using Telstar Lyoquest Lyophillizer. Additionally, they were stored in amber colored glass vials with proper labelling to prevent moisture absorption and light damage. The labelled glass vials containing extracts were then subsequently used for qualitative and quantitative phytochemical estimation (Aspé & Fernández, 2011).



Seaweed *Turbinaria decurrens* | Powdered *Turbinaria decurrens* | Soxhlet's extraction assembly | Extraction process using three solvents Acetone, Aqueous & Methanol | Drying of extracts using Rota Evaporator

Figure 6: Extraction of phytochemical constituents by Hot percolation method using Soxhlet Extraction assembly and drying of solvent extracts using Rotary evaporator.

3.5 PHYTOCHEMICAL SCREENING

3.5.1 Qualitative Estimation: The qualitative phytochemical analysis included estimation of alkaloids, anthraquinones, coumarins, glycosides, flavonoids, phenol, saponins, steroids, tannins and terpenoids by following methods (Edeoga *et al.*, 2005; Patil, 2020).

a) **Detection of Alkaloids:** For determination of alkaloids in the seaweed extract sample, the first step was to prepare Mayer's reagent by dissolving 0.355gram of mercury (II) chloride and 5grams of potassium iodide in water. 2mL of seaweed extract was mixed with 2mL of concentrated HCl. Few drops of this mixture were combined with the seaweed extract. The resulting filtrate, upon treatment exhibited turbidity or precipitation, along with a green color signifying the presence of alkaloids.

b) **Detection of Anthraquinones:** One gram of seaweed extract was mixed into 20mL chloroform inside a dry test tube then boiled for five minutes inside a steam bath. The resulting percolate underwent cooling, with filtration first. After that, the percolate was mixed with an equal volume of 10% ammonia solution. Agitating the mixture for noting a bright pink hue verified anthraquinones detection in the upper aqueous layer. A reference solution was prepared by mixing 10mL of 10% ammonia solution with 5mL of chloroform.

c) **Detection of Coumarins:** A few drops of alcoholic sodium hydroxide was added to 2mL of the test solution. Appearance of yellow color indicated the presence of coumarins (Yahia *et al*., 2021).

d) **Detection of Glycosides:** A solution was formulated by combining 2mL of the extract with glacial acetic acid, a drop of ferric chloride, and concentrated sulfuric acid. The appearance of a reddish-brown pigment at the interface of two layers, accompanied by a blue green tint in the upper layer, indicated the presence of glycosides.

e) **Detection of Flavonoids:** The presence of flavonoids was determined by boiling 0.5grams of extract with distilled water and filtered. A few drops of a 10% ferric chloride solution were then added to 2mL of the filtrate. The appearance of a green-blue or violet color suggested the presence of phenolic hydroxyl groups.

f) **Detection of Phenols:** To assess phenolic compounds, 1mL of the extract was mixed with 2mL of deionized water, followed by the addition of 0.5mL of sodium carbonate

and 0.5mL of Folin-Ciocalteau's reagent. The presence of phenols was indicated by a blue-green coloration.

g) **Detection of Saponins:** In a test tube, dissolve 0.5grams of seaweed in 2mL of heated water. The seaweed solution was allowed to settle down and then shaken vigorously to ensure thorough mixing. The presence of foam indicated the detection of saponins.

h) **Detection of Steroids:** The presence of steroids was determined by combining 2mL of the extract, 2mL of chloroform, and 2mL of concentrated sulfuric acid. The emergence of a cherry color in the lower chloroform layer signified the existence of steroids.

i) **Detection of Tannins:** 0.5 grams of seaweed extract powder was heated in 20mL of purified water within a test tube, and filtered. 1mL of the filtered extract was accompanied with 1mL of 5% $FeCl_3$. The appearance of a brownish-green color indicated the presence of tannins.

j) **Detection of Terpenoids:** The presence of terpenoids was confirmed by mixing 1mL of extract with 2mL of chloroform and gently overlaid with 1.5mL of concentrated sulphuric acid. The development of reddish-brown color at the interface signified the existence of terpenoids.

3.5.2 Quantitative estimation: The quantitative analysis of those metabolites which were present qualitatively was carried out to measure their respective concentrations in all the seaweed extracts.

a) Total Coumarin Content: Total coumarin content was estimated following the method described by Vianna *et al*., 2011 with slight modifications. 10mg of each seaweed extract was dissolved in 1mL of a methanol: acetone mixture (1:1, v/v) in a 2mL microcentrifuge tube. The mixture was vortexed thoroughly and allowed to stand at room temperature (30°C) for 30 minutes to ensure complete extraction. Following incubation, the tubes were centrifuged at 5000rpm for 10 minutes using a tabletop centrifuge. The resulting supernatant was carefully decanted without disturbing the pellet. The volume was then adjusted to 2mL using the same methanol: acetone solvent, and the solution was vortexed again to ensure homogeneity. The clear supernatant was transferred into a cuvette

and absorbance was measured at 327nm using a UV-visible spectrophotometer. A solvent blank was used as a reference containing methanol:acetone. Total coumarin content was quantified using standard calibration curve prepared with esculin.

b) Total Glycoside Content: Cardiac glycoside content in each seaweed extract was quantitatively determined based on the method described by Solich *et al*., 1992, with slight modifications. For the assay, 100µL of each extract was mixed with 1mL of freshly prepared Baljet's reagent composed of 95mL of 1% picric acid and 5mL of 10% sodium hydroxide (NaOH). The mixture was allowed to stand at room temperature for 1 hour to complete the reaction. Following incubation, the reaction mixture was diluted with 2mL of distilled water. The absorbance of the resulting solution was measured at 495nm using UV-VIS spectrophotometer. The concentration of cardiac glycosides was calculated from a standard curve expressed as milligrams of digoxin as equivalent per gram of dried extract (mg digoxin/g extract).

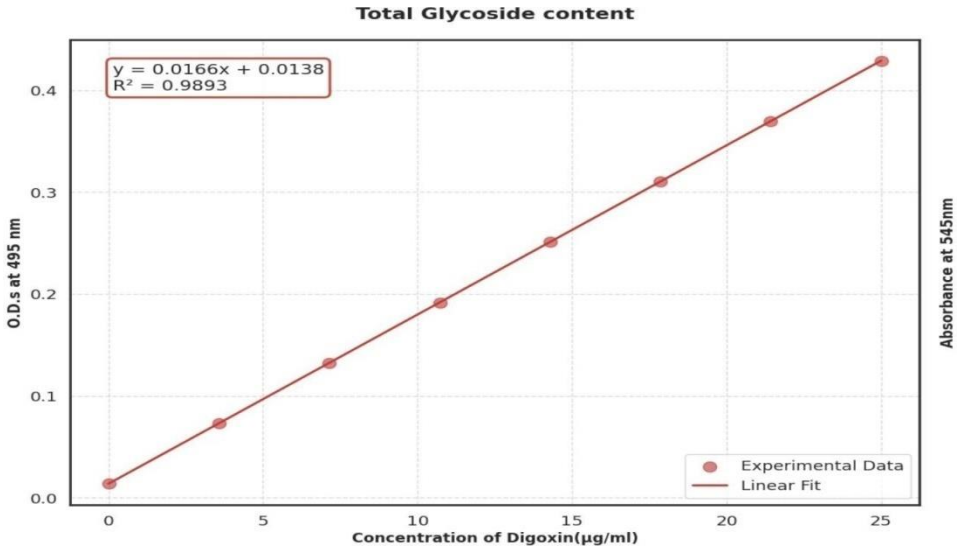


Figure 7: Standard Digoxin curve for Cardiac Glycoside estimation

c) Total Phenolic Content: The total phenolic content was determined spectrophotometrically, following the Folin-Ciocalteau method as described by Singleton *et al*., 1999. In a screw cap test tube, 200µL from extract was taken, and mixed with 1mL of Folin-Ciocalteau reagent (diluted 1:1 with water) and 1mL of a 7.5% sodium carbonate

solution. The tubes were vortexed and incubated for 2 hours, after which the absorbance was measured at 760nm with a Beckman spectrophotometer (USA). The total phenolic content was reported as gallic acid equivalents (GAE) in milligrams per gram of dry material.

$$\text{Total Phenolic Content} = \frac{\text{Amount of Gallic Acid (mg)}}{\text{Mass of Sample (g)}} \times \text{Volume of Extract (mL)}$$

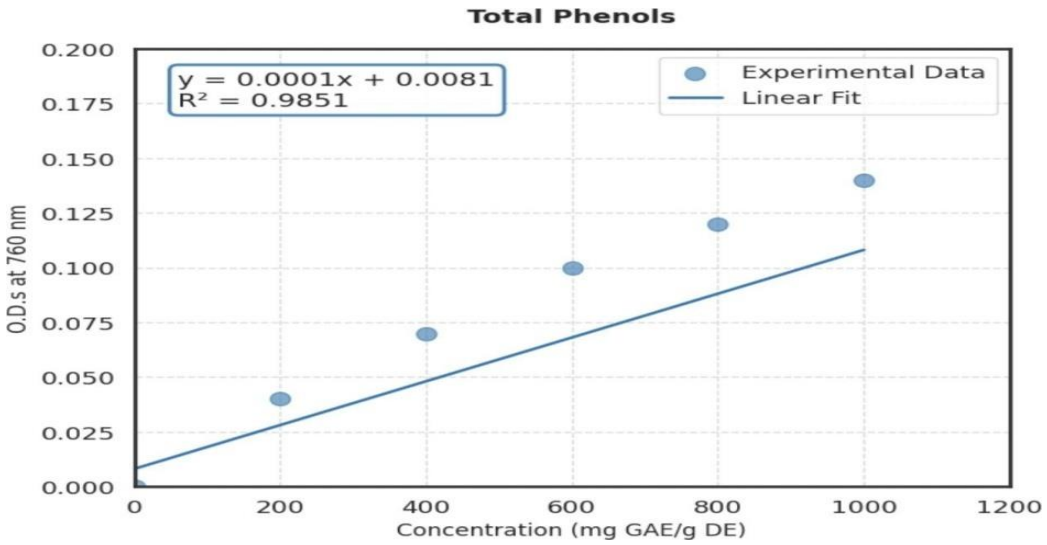


Figure 8: Standard Gallic acid curve for estimation of Total Phenols

d) Total Tannin Content: The total tannin content of the seaweed extract was determined by the method described by Siddhuraju *et al*., 2007. A volume of 500µL of the seaweed extract was mixed with 100mg of polyvinyl polypyrrolidone and 500µL of distilled water in a separate test tube. The mixture was incubated at 4°C for 4 hours. The centrifugation was done at 5000rpm for five minutes and the supernatant was separated. This supernatant contained only simple phenolics, as the tannins had precipitated with the polyvinyl polypyrrolidone. The concentration of phenolics in the supernatant was measured at 725 nm and expressed as gallic acid equivalents (GAE) on a dry matter basis. The tannin content was calculated using the following formula:

Total Tannin Content = Total Phenol-Free Phenol

e) Total Terpenoid Content: The total terpenoids were quantified using the method established by Ghorai *et al.*, 2012. A volume of 200µl aliquot of methanol was taken, to which a known quantity of sample (20 mg) was added along with 1.5mL of chloroform in each 2mL micro-centrifuge tube. The sample mixture was vortexed thoroughly and allowed to stand for 3 minutes. Subsequently, 100µL of concentrated sulfuric acid (H_2SO_4) was added to each 2mL micro-centrifuge tube. The tubes were then incubated at room temperature (30°C) for a duration of 1.5 hours to 2 hours in the dark. At the end of the incubation period, a reddish-brown precipitate formed in each assay micro-centrifuge tube. Decant all the supernatant carefully. Following this, 1.5mL of 95% (v/v) methanol was introduced and vortexed thoroughly until the precipitate was completely dissolved in the methanol. The sample was then transferred from the assay tube to a cuvette, and the absorbance was measured at 545nm, using 95% (v/v) methanol as the blank. The total terpenoids were calculated using a standard curve derived from the blank corrected absorbance of Linalool standard.

$$\text{Total Terpenoid Content} = \frac{\boldsymbol{Concentration\ of\ Linalool \times Amount\ of\ Terpenoid\ extracted}}{\boldsymbol{Weight\ of\ extract}} \times 100$$

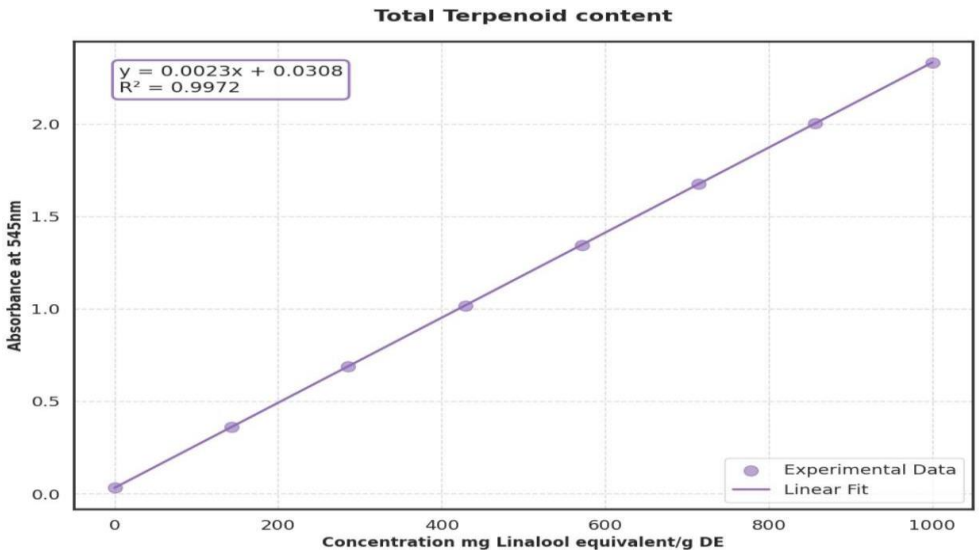


Figure 9: Standard Linalool curve for Total Terpenoid Content

3.6 In-vitro Antioxidant Assay: The antioxidant ability of different extracts was estimated using four methods namely ABTS (2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), DPPH (1,1-diphenyl-2-picrylhydrazyl), FRAP (Ferric Reducing Antioxidant Power) and LPO (Lipid Peroxidation) antioxidant assays.

3.6.1 ABTS Assay: The different seaweed extract's antioxidant capacity was assessed utilizing the ABTS decolorization method (Re *et al*., 1999). A concentration of 7mM ABTS solution in water was prepared, and ABTS radical cation ($ABTS^{\bullet+}$) was generated by mixing it with 2.45mM potassium persulfate. The mixture was then allowed to stand in the dark for 12-16 hours. The ABTS*+ solution was further diluted with ethanol to achieve an absorbance of 0.7 (±0.02) at 734nm, and subsequently equilibrated at 30°C. Furthermore, 50µL of a seaweed extract (20mg/mL) and 2mL of the diluted ABTS*+ solution was created, for the measurement of absorbance at 734nm exactly after 1 minute after initial mixing and up to 6 minutes after the components were combined. Solvent blanks were utilized in each experiment, and measurements were conducted in triplicate using concentrations of 0.2, 0.4, 0.6, 0.8, and 1mg/mL to enhance reliability. The following calculations were used:

$$\textbf{Percent Inhibition (PI)} = \frac{\boldsymbol{Absorbance\ Control - Absorbance\ Test}}{\boldsymbol{Absorbance\ Control}} \times 100$$

3.6.2 DPPH Assay: The DPPH radical scavenging activity was determined by the method described by (Gadow *et al*., 1997). A volume of 2mL of a 6×10^{-5}M methanolic solution of DPPH was combined with 50µL of 20mg/mL seaweed sample solution. The absorbance was measured at 515nm using a spectrophotometer over a period of 16 minutes at room temperature. The scavenging effect was graphed against time, and the percentage of DPPH radical scavenging capacity of the sample was determined from the absorbance value recorded at the end of the 16 minutes interval. All measurements were conducted in triplicate. The percentage inhibition of the DPPH radical by the samples was computed using the formula provided by Yen *et al*., 1994.

$$\textbf{Percent Inhibition (PI)} = \frac{\boldsymbol{Absorbance\ Control - Absorbance\ Test}}{\boldsymbol{Absorbance\ Control}} \times 100$$

3.6.3 FRAP Assay: The FRAP assay was performed in accordance to the methodology outlined by Benzie & Strain, 1996, with certain modifications. The stock solutions included a 300 mM acetate buffer (3.1g of $C_2H_3NaO_2 \cdot 3H_2O$ and 16mL of $C_2H_4O_2$, adjusted to pH 3.6), a 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40mM HCl, and a 20 mM $FeCl_3 \cdot 6H_2O$ solution. A fresh working solution was prepared by mixing 25mL of acetate buffer, 2.5mL of TPTZ solution, and 2.5mL of $FeCl_3 \cdot 6H_2O$ solution, which was subsequently heated to 37°C before use. seaweed extracts (150µL) were incubated with 2850µL of FRAP solution in the dark for a duration of 30 minutes. The absorbance of the resulting-colored product, a ferrous tripyridyltriazine complex, was measured at 593nm.

$$\textbf{Percent Inhibition (PI)} = \frac{\textbf{Absorbance Control} - \textbf{Absorbance Test}}{\textbf{Absorbance Control}} \times \mathbf{100}$$

3.6.4 Lipid Peroxidation Assay: Lipid peroxidation assay was conducted in according to the method described by Ohkawa *et al*., 1979. In this assay, Ferrous Sulphate ($FeSO_4$) ascorbate system in sample was measured utilizing the thiobarbituric acid reactive substances (TBARS) technique. The reaction mixture consists of 0.1 mL of seaweed extract in Tris-HCl buffer (20 mM, pH 7.0; KCl 30mM; $FeSO_4$ $(NH_4)_2SO_4 \cdot 7H_2O$ 0.06mM) along with varying amounts of synthesized peptides, culminating in a final volume of 0.5mL. The mixture was incubated at 37°C for one hour. Following incubation, 0.4mL was extracted and analyzed using sodium dodecyl sulfate (8.1%), thiobarbituric acid (0.8%), and trichloroacetic acid (20%). The mixture underwent heating, cooling with tap water and 5.0mL of mixture of n-butanol and pyridine were added and centrifuged. After centrifugation the absorbance of butanol-pyridine layer was observed at 532nm.

$$\textbf{Percent Inhibition (PI)} = \frac{\textbf{Absorbance Control} - \textbf{Absorbance Test}}{\textbf{Absorbance Control}} \times \mathbf{100}$$

3.7 *IN-VITRO* ANTIDIABETIC ACTIVITY: *In-vitro* antidiabetic evaluation refers to the potential effect of certain compounds on diabetes under controlled laboratory conditions. The assays performed to evaluate antidiabetic activity for seaweed were; α (alpha) amylase and glucosidase inhibitory activity.

3.7.1 α -Amylase Inhibitory Activity: The assay was conducted following method of Mccue *et al*., 2004. Initially 250µL of sample was added to a tube, followed by the addition 250µL of 0.02M sodium phosphate buffer (pH 6.9) that contained α-amylase solution (0.5mg/mL). This mixture was pre-incubated at 25°C for 10 minutes, after which 250µL of a 1 % starch solution in 0.02M sodium phosphate buffer was introduced at specific time intervals. The mixture was further incubated at 25°C for an additional 10 minutes. The reaction was halted by the addition of 500µL of dinitrosalicylic acid (DNSA) reagent. Subsequently, the tubes were placed in boiling water for 5 minutes and then allowed to cool at room temperature. The reaction mixture was diluted with 5mL of distilled water and the absorbance was measured at 540nm using spectrophotometer.

A control was prepared using the same procedure, substituting the sample with distilled water. Additionally, the acarbose standard was also processed in the same manner.

$$\textbf{Percent Inhibition (PI)} = \frac{\boldsymbol{Absorbance\ Control - Absorbance\ Test}}{\boldsymbol{Absorbance\ Control}} \times 100$$

3.7.2 α-Glucosidase Inhibitory Activity: The technique described by Watanabe *et al*., 1997 was employed for the determination. Yeast α-glucosidase (0.7 U) was dissolved in a 100mM phosphate buffer (pH 7.0) that contained 2g/L bovine serum albumin, 0.2g/L of NaN3 and 5mM of p-nitrophenyl- α-D-glucopyranoside in the same buffer, which served as the enzyme and substrate solution, respectively. A total of 1000µL of the enzyme solution was combined with 100µL of test sample at varying concentration, and the absorbance at 405nm was recorded using a spectrophotometer (UV1800 Shimadzu, Japan). Following a 5-minute incubation, 50µL of the substrate solution was introduced and incubated for an additional 5 minutes. The change in absorbance from the initial measurement was recorded and inhibitory activity was determined as a percentage of the blank control.

$$\textbf{Percent Inhibition (PI)} = \frac{\boldsymbol{Absorbance\ Control - Absorbance\ Test}}{\boldsymbol{Absorbance\ Control}} \times 100$$

3.8 CYTOTOXICITY STUDY: Cytotoxicity assays are tests conducted in laboratories to assess the possible toxic effects of various substances on living cells. When applied to

seaweeds, these assays are instrumental in determining if seaweed extracts can kill or impede the growth of cancer cells or other types of cell lines. The assay conducted was MTT (3-(4,5-dimethylthiazole-2-yl)-2, 5-diphenyltetrazolium bromide) assay.

3.8.1 MTT assay (3-(4,5-dimethylthiazole-2-yl)-2, 5-diphenyltetrazolium bromide): The MTT assay involves three steps preparation of 3T3 fibroblast cell lines, seeding of cells onto a 96-well plate and assessment of cytotoxicity. On day one, a subculture of 3T3 cells in Dulbecco's Modified Eagle's Medium (DMEM) was trypsinized individually, following the removal of the culture medium. 25mL of DMEM containing 10% fetal calf serum (FCS), was introduced to disaggregated cells in the flask. The cells were suspended in the medium through gentle pipetting, resulting in a homogenized cell suspension. 1mL of the homogenized cell suspension was introduced into each well of a 24-well culture plate, accompanied by samples of acetone, aqueous and methanol at varying concentrations, including 0µg/mL (control), 12.5µg/mL, 25µg/mL, 50µg/mL, 100µg/mL and 200µg/mL. The plate was then incubated at 37°C in a humidified carbon dioxide (CO_2) incubator with a concentration of 5% CO_2. Following 48 hours of incubation, the cells were examined using an inverted tissue culture microscope. The assay was conducted utilizing MTT. It is metabolized by mitochondrial succinate dehydrogenase and reductase present in viable cells, resulting in the formation of a quantifiable purple-colored formazan product. The production of formazan is directly proportional to the number of viable cells and inversely proportional to the level of cytotoxicity. Following the treatment, the medium containing the extract was substituted with fresh basal medium, and 100µL of MTT (1mg/mL) was added to the wells, which were then incubated for 3 hours at room temperature. The content of all wells, were subsequently removed using a micropipette, and 100µL of DMSO was introduced to dissolve the formazan crystals. The absorbance was measured using a Robonic Readwell Touch microplate reader at a wavelength of 570nm (Mosmann, 1983). The percentage of cell viability was estimated by given equation.

$$\%\text{age Viable cells} = \frac{\boldsymbol{Absorbance\ of\ sample - Absorbance\ of\ blank}}{\boldsymbol{Absorbance\ of\ control - Absorbance\ of\ blank}} \times 100$$

3.9 ANIMAL EXPERIMENTATION:

3.9.1 Procurement of Animals: Wistar rats, each weighing between 150-220 grams were sourced from the Central Animal House Facility NIPER Mohali Punjab, India, with prior approval from the Institutional Animal Ethical Committee of Khalsa College of Pharmacy Amritsar Punjab, India (Approval No. IAEC/KCP/2024/008). The animals were kept under standard conditions, maintaining a temperature of 24°C ± 2°C and a 12-hour light-dark cycle, with unrestricted access to both food and water. All safety protocols and regulations established by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) were meticulously followed to ensure the well-being of the animals.

3.9.2 Acute Toxicity Study: To evaluate acute toxicity over a period of 14 days twelve healthy female Wistar rats, were randomly divided into four groups (n=3 per group). The study compiled with the OECD 423 guidelines for acute toxicity studies (Annexure 2b) (OECD Guidelines, 2002). Before commencing the experiment, the body weight (BW) of each rat was recorded individually to determine the suitable dosage of the extract. The rats were divided into groups based on their weights: Group-1 (G 1) Negative control, which received 0.9% normal saline (NS) at a dosage of 10mg/Kg body weight (BW); Group-2 (G 2) *T. decurrens* methanolic extract at 50mg/Kg BW; Group-3 (G 3) *T. decurrens* methanolic extract at 300mg/kg BW; and Group-4 (G 4) *T. decurrens* methanolic extract at 2000mg/kg BW. The determination of body weight was done using a weighing balance. The weight was measured prior to dosing (day 0), weekly (day 7) and subsequently recorded until the experiment concluded (day 14). Observations concerning the overall behavior of the rats, instances of lethality, and any unusual clinical signs were recorded on the day of dosing, four hours post administration and subsequently over a 24-hour period and continued for an additional 13 days. (Caroline *et al*., 2018). At the conclusion of acute toxicity study blood samples were collected to determine hematological and biochemical profile of all groups. The animals were sacrificed and vital organs like liver and kidney were removed, washed in normal saline and fixed in buffered formalin for histopathological examination.

Figure 10: Animal House of Khalsa College of Pharmacy Amritsar

Figure 11: Animals housed in polypropylene cages

3.9.2.1 HEMATOLOGICAL & BIOCHEMICAL INVESTIGATIONS: At the conclusion of acute oral toxicity testing, blood samples were obtained from the animals for both hematological and biochemical analysis. The rats were anesthetized using 1.9% inhaled diethyl ether (Essa *et al*., 2020) and retro-orbital puncture was performed using a sterile hematocrit capillary tube for the collection of blood (Van Herck *et al*., 2001). The blood sample from each animal was collected into three distinct tubes: Dipotassium Ethylenediamine Tetraacetate (K_2EDTA), Fluoride, and Clot activator vial. The blood stored in the K_2EDTA tube was employed for hematological assessments, including Hemoglobin (Hb), White Blood Cell count (WBC), Differential Leucocyte Count (DLC), Red Blood Cell Count (RBC), Packed Cell Volume (PCV), and Platelets, utilizing an Automated Hematology Analyzer (Mindray BC-5150) (Kampfmann *et al*., 2012). Furthermore, the blood samples in the clot vials were left undisturbed for 2 hours and then centrifuged at 5000rpm for 10minutes to obtain serum for biochemical analysis (Kharchoufa *et al*., 2020) focusing on Fasting Blood Glucose (GOD/POD method) (Trinder, 1969), Renal Function Tests including Blood Urea Nitrogen (BUN) (GLDH-UREASE method) (Fawcett *et al*., 1960) Serum Creatinine Jaffe's reaction (Bonsnes *et al*., 1945). Lipid profile was assessed by determining Total Cholesterol (Richmond, 1973), High Density Lipoproteins (HDL) (Yeates *et al*., 1979), Serum Triglycerides (TG) (Fossati *et al*., 1982). Additionally Low-Density Lipoproteins (LDL) and Very Low-Density Lipoproteins (VLDL) were calculated

by Friedewald's equation (Vargas *et al*., 2021). Liver parameters included determination of enzymes Serum Glutamate Oxaloacetate Transaminase (SGOT) and Serum Glutamate Pyruvate Transaminase (SGPT) (Bergmeyer *et al*., 1986). All biochemical investigations were performed following the protocols outlined in the respective kits and results were generated using a semi-automated biochemistry analyzer (ERBA CHEM-5).

Figure 12: Blood sample collection from retro orbital plexus

3.9.2.2 HISTOPATHOLOGICAL INVESTIGATION: After blood collection, the rats were euthanized through cervical dislocation and essential organs such as liver and kidney were removed for the examination of pathological alterations. The organs were cleaned in normal saline. The weight of the organs was recorded prior to their transfer to the histopathology laboratory. Relative organ weight (ROW) was calculated using the formula (Pinto *et al*., 2025).

ROW (%) = (Organ weight/body weight) ×100

Figure 13: Animal dissection

Figure 14: Weight of Kidney (grams)

The histological processing of these tissues commenced with fixation and dehydration, followed by paraffin embedding, sectioning and staining. Hematoxylin and Eosin (H & E) stain was applied to the slides (Bhardwaj *et al*., 2021). The stained slides were subsequently examined under the (Olympus CH20i) research microscope.

Figure 15: Organ stored in formalin and tissue blocks

3.9.3 PHARMACODYNAMIC STUDIES: These studies included the biochemical and physiological effects of drugs on the body and their mechanism of action. The principle of pharmacodynamic studies is essential in the design of new drugs.

3.9.3.1 Experimental Protocol; Diabetes induction in Wistar rats: The experimental design's first step included induction of diabetes in Wistar rats. To initiate diabetes the rats underwent an overnight fasting followed by intraperitoneal injection of streptozotocin (STZ) (55mg/kg BW) (Padugupati *et al*., 2021) (Mohan *et al*., 2013). Prior to use, the STZ was dissolved in a 0.1 M freshly prepared sodium citrate buffer with a pH of 4.5, which was rendered isotonic through the incorporation of 0.25M NaCl. A 5% glucose solution was administered for two consecutive days to avert drug-induced hypoglycemic shock. The fasting blood sugar levels were determined before and 72 hours after the injection. The rats that exhibiting fasting blood glucose levels >250mg/dl were selected for further examination (Mohan *et al*., 2013). Based on the preliminary *in-vitro* assays viz antioxidant, antidiabetic and cytotoxic findings among acetone, aqueous and methanolic extract of *Turbinaria decurrens*; the therapeutically active methanolic extract was for *in-vivo* antidiabetic evaluation in streptozotocin induced Wistar rats. The animals were divided into the following experimental groups:

- **Group 1:** Control (non-diabetic) (10mL/kg body weight Normal Saline)
- **Group 2:** Streptozotocin (untreated-diabetic group) (55mg/kg body weight) (Padugupati *et al*., 2021b) (Mohan *et al*., 2013)
- **Group 3:** Streptozotocin + Metformin (10mg/kg body weight)
- **Group 4:** Streptozotocin + Low dose (100mg/kg body weight) Methanolic extract *Turbinaria decurrens (TD)*
- **Group 5:** Streptozotocin + Medium (Med) dose (200mg/kg body weight) Methanolic extract *Turbinaria decurrens (TD)*
- **Group 6:** Streptozotocin + High dose (400mg/kg body weight) Methanolic extract *Turbinaria decurrens (TD)* (Kamboj *et al*., 2013).

3.9.3.2 Body weight measurements and blood sample collection: The body weight of each rat was measured using a weighing balance on day 1,14 and 28 to monitor the changes in weight during the treatment (Elijah, 2019). Baseline fasting blood samples were collected by retro-orbital puncture using sterile capillary tube from all rats in K_2EDTA, Fluoride and Clot activator tube before the experiment began and 28th day of the treatment. The sample in K_2EDTA vial was kept for hematological investigation, the sample in fluoride vial and clot activator vial was centrifuged and serum/plasma (Kharchoufa *et al*., 2020) was obtained for biochemical investigations.

3.9.3.3 Hematological investigation: The K_2EDTA vials were kept on a blood tube rotator for mixing the blood sample. Hematological parameters *viz* Hemoglobin, Total Leukocyte Count (TLC), Differential Leukocyte Count (DLC) (Neutrophils, Lymphocytes), Red Blood Cell Count (RBC) and Platelet count were determined as per standard protocol in an Automated Complete Blood Cell (CBC) Analyzer (Mindray 5-part) (Kampfmann *et al*., 2012).

Figure 16: CBC Analyzer (Mindray)

3.9.3.4 Biochemical Estimations: The biochemical investigation included blood glucose (Trinder, 1969), total cholesterol (Richmond, 1973), serum triglycerides (Fossati *et al*., 1982), high density lipoproteins (HDL) (Yeates *et al*., 1979), low density lipoproteins (LDL), very low density lipoproteins (VLDL) (Vargas et al., 2021), serum glutamate

oxaloacetate (SGOT), serum glutamate pyruvate transaminase (SGPT) (Bergmeyer *et al*., 1986), blood urea (Fawcett *et al*., 1960), serum creatinine (Bonsnes *et al*., 1945) were measured using commercial kits according to manufacturer protocols. Analysis was conducted with a Semi-Automated Biochemistry Analyzer (ERBA-CHEM 5).

Figure 17: Biochemistry Analyzer (ERBA CHEM-5 plus)

➢ **Estimation of Blood Glucose:** Concentration of glucose was assessed through GOD/POD (Glucose oxidase peroxidase) method. Glucose oxidase catalyzes the conversion of glucose to gluconic acid producing hydrogen peroxide, which reacted with 4-aminoantipyrine and phenol in the presence of peroxidase to form a red quinonimine dye. Three tubes were taken for the procedure and labelled as test (T), standard (S), blank (B). To all the three tubes 1mL of glucose reagent was added and 10µL of plasma was added to the tube labelled as T, 10µL of distilled water to the tube B and 10µL of glucose standard to the tube S. The tubes were then incubated at 37°C for 10 minutes. The absorbance of S and T against B was measured at 505nm within 60 minutes.

Calculation:

$$\text{Glucose (mg/dl)} = \frac{Absorbance\ of\ test\ (T)}{Absorbance\ of\ standard\ (S)} \times Concentration\ of\ standard\ (100)$$

➢ **Estimation of Total Cholesterol:** The total cholesterol estimation was performed by the method of cholesterol oxidase-peroxidase aminoantipyrine (CHOD/PAP). Cholesterol esterase hydrolyzes cholesterol esters to liberate free cholesterol, which is then oxidized to cholest-4-en-3-one by cholesterol oxidase. In the presence of peroxidase, hydrogen peroxide reacts with 4-aminoantipyrine and phenol to produce a red dye, intensity of which is measured at 505nm. In the assay procedure three tubes were taken and labelled as T, S and B. To all the three tubes 1mL of working reagent was added. To the tube labelled as T, S and B add 10µL of serum, cholesterol standard and distilled water respectively. Mix the contents of each tube separately and incubate for 5 minutes at 37°C. Measure the absorbance of T and S against B.

Calculation:

$$\text{Total Cholesterol (mg/dl)} = \frac{Absorbance\ of\ test\ (T)}{Absorbance\ of\ standard\ (S)} \times Concentration\ of\ standard\ (200)$$

➢ **Estimation of Triglycerides:** Triglycerides (TG) were determined by the glycerol phosphate oxidase peroxidase anti-phenazon (GPO/PAP) method. TG were hydrolyzed by lipase to yield glycerol which is then phosphorylated by glycerol kinase and oxidized to dihydroxyacetone phosphate and hydrogen peroxide. In the presence of peroxidase, hydrogen peroxide oxidizes the chromogen to a red compound, which is then measured at 510nm. To estimate triglycerides three tubes labelled as T, S & B were taken and 1mL of working reagent (mixture of enzyme reagent 1 & 2) was added to all three tubes. 10µl of serum was added to tube T, 10µl of standard was added to the tube S and 10µl of distilled water was added to tube B. The tubes were incubated for 10 minutes at 37°C.

Calculation:

$$\text{Triglycerides (mg/dl)} = \frac{Absorbance\ of\ test\ (T)}{Absorbance\ of\ standard\ (S)} \times Concentration\ of\ standard\ (200)$$

➢ **Estimation of HDL Cholesterol:** The HDL cholesterol was estimated by the method of PEG/CHOD-PAP method. The first step was to obtain supernatant by mixing

precipitating reagent (L3) (provided in the kit) with sample. Furthermore, centrifugation was done to obtain clear supernatant where all the VLDL and LDL are precipitated. The remainder supernatant containing HDL was assayed by mixing 0.05mL of supernatant (T), standard (S), and distilled water (B) with 1mL of working reagent (mixture of reagent namely L2 & L1) and incubating at 37°C for 5 minutes.

Measure the absorbance of test and standard against blank at 505nm.

Calculation:

$$\text{HDL Cholesterol (mg/dl)} = \frac{Absorbance\ of\ test\ (T)}{Absorbance\ of\ standard\ (S)} \times Concentration\ of\ standard\ (25) \times 2$$

where 2 is the dilution factor due to protenization step.

- **Estimation of LDL and VLDL cholesterol:** The LDL and VLDL levels were calculated using Friedwald's formula.

Calculation:

$$\text{LDL Cholesterol (mg/dl)} = Total\ Cholesterol - (\frac{Triglycerides}{5}) - (HDL\ Cholesterol)$$

$$\text{VLDL Cholesterol (mg/dl)} = \frac{\boldsymbol{Triglycerides}}{\mathbf{5}}$$

- **Estimation of Urea:** The determination of urea in serum was performed by the urease-glutamate dehydrogenase (GLDH) method. Urease hydrolyzed urea to ammonia and carbon dioxide which further reacted with α ketoglutarate and NADH to form glutamate and NAD. The oxidized NADH to NAD is measured as a decrease in absorbance in a fixed time which indicated the concentration of urea in the sample. The estimation of urea included preparation of working reagent by mixing together 4 parts of enzyme reagent (L1) and 1 part of starter reagent (L2). The next step included measurement of absorbance of standard and test at 340nm. For this take two test tubes labelled as T (test) and S (standard). Add 1.0mL of working reagent in both the tubes and add 10µL of serum and standard in tube T and S with the help of micropipette. Mix well and read the initial absorbance A_1 for the standard and test after exactly 30 seconds.

Read another absorbance A_2 of the standard and test exactly after 60 seconds. Calculate the change in absorbance ΔA for both standard and test.

Calculation: (Urea in mg/dl = $\frac{\Delta AT}{\Delta AS} \times 40$) where $\Delta AS = A_2S - A_1S$, $\Delta AT = A_2T - A_1T$

- **Estimation of Creatinine:** The estimation of creatinine in serum was done by the alkaline picrate method. Picric acid in an alkaline medium reacted with creatinine to form orange colored complex with alkaline picrate. The intensity of the color formed was directly proportional to the concentration of creatinine in the sample and absorbance was measured at 520nm. The first step of estimation included deproteinization of sample in which 2mL of picric acid was taken in a tube along with 20µL of test sample. Mixed well and centrifuged at 2500-3000rpm for 10 minutes to obtain a clear supernatant. Next step was the development of color in which three tubes labelled as T (test), S (standard) and B (blank) were taken and 1mL of picric acid was added to tube S and B only. To the tube labelled T, 1.1mL of supernatant was added. To the tube labelled as S and B 0.1mL of standard and distilled water were added respectively. Finally buffer reagent (L2) 0.1mL was added to all three tubes and kept at room temperature for 20 minutes. The absorbance of T and S was taken against B.

  Calculation:

$$\text{Creatinine in mg \%} = \frac{Absorbance\ of\ Test}{Absorbance\ of\ Standard} \times 2.0$$

- **Estimation of SGOT:** The SGOT in serum was performed by IFCC method. The principle involves the catalyzes of L-aspartate and α-ketoglutarate to oxaloacetate and glutamate. The oxaloacetate and NADH oxidation to NAD was measured spectrophotometrically at 340nm. Prepare the working reagent as per the instructions given in literature. To a tube labelled as T 1mL of working reagent and 100µL of serum was added. They were allowed to mix properly and transferred to a cuvette and stop watch was started immediately. The first reading was taken at 60 seconds and three more readings with 30 seconds interval.

  Calculation: Activity of SGOT (IU/L) = Δ Absorbance/minute × 1749

- **Estimation of SGPT:** The IFCC method was followed to determine SGPT in serum. The principle is based on the conversion of NADH to NAD during the conversion of pyruvate to L-malate which is proportional to the concentration of SGPT and was measured at 340nm. To determine the concentration of SGPT a mixture of 1mL of working reagent and 100µL of serum was taken in a test tube and mixed thoroughly. Further, it was transferred to a cuvette and stopwatch was started immediately. The first reading was taken at 60 seconds interval and subsequently three more reading were with 30 seconds interval.

  Calculations: Activity of SGPT (IU/L) = Δ Absorbance/minute × 1768

3.9.3.5 Histopathological Investigations: The histopathological investigations included the processing of tissue along with staining after collecting blood samples for biochemistry. According to the procedure of Wadaan 2009, the animals were sacrificed through cervical dislocation. The liver, kidney and pancreas were dissected from the rat's body, washed in normal saline, weighed properly and then fixed in 10% neutral buffered formalin before being processed for histopathology.

3.9.3.5.1 Processing of tissue: The tissue specimens were subjected to dehydration with increasing concentration of ethanol, so that tissue can be prepared for embedding by removing water. The next step was clearing in which ethanol was replaced by xylene which make tissue transparent and removes ethanol to make room for paraffin wax. Finally, tissues were transferred to paraffin embedding molds for obtaining blocks of tissue for section cutting. Sections 6µm thickness were then cut using a rotary microtome.

3.9.3.5.2 Staining Process (Hematoxylin and Eosin Stain): The H & E stain involved a series of steps to stain the sections onto a slide. The sections were fixed onto a clean glass slide and it was placed in xylene for deparaffinization. The next step involved rehydration by decreasing concentration of ethanol. The slides were further stain Harry's hematoxylin for 5-10 minutes and rinsing in running tap water. Dip the slides in Eosin Y for 30 seconds and then pass through increasing concentration of ethanol. Place in xylene and mount using DPX and cover with cover slip carefully (Drury, 1980).

3.9.3.5.3 Microscopic examination and preparation of homogenate from tissue specimen: The stained sections were then viewed under Olympus research microscope Ch 20i for observing the morphological changes if any. Furthermore, the dissected tissues; liver, kidney and pancreas were rinsed with ice cold normal saline to remove blood and were preserved in deep freezer (-20°C) for homogenate preparation. The liver, kidney and pancreas tissues (100mg) were cut up into small pieces, added to 1mL of 0.1 M phosphate buffer (pH 7.4) and homogenized using a Teflon tissue homogenizer (Remi). The tissue homogenates were then centrifuged at 15000g at 4°C for 30 minutes.

Furthermore, supernatant so obtained was filtered and preserved at -20°C until it was analyzed (Ahmed *et al*., 2018).

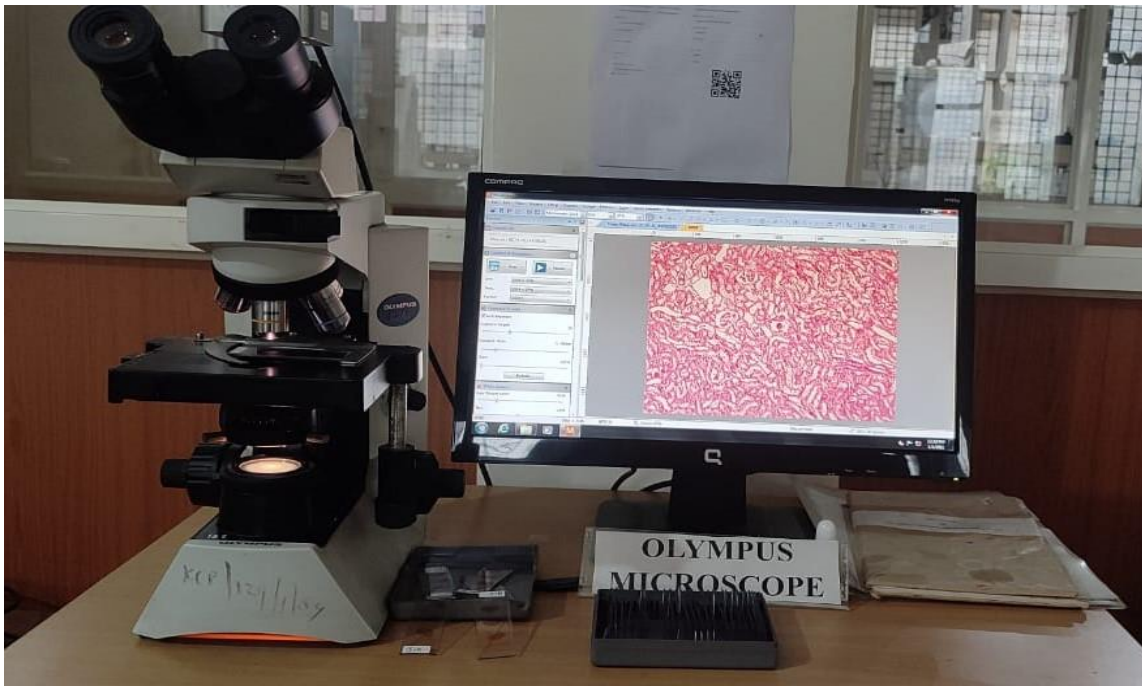


Figure 18: Olympus Microscope

3.9.3.6 *In-vivo* Antioxidant assay: *In-vivo* antioxidant assays included catalase test, glutathione peroxidase, superoxide dismutase and malondialdehyde assay.

Figure 19: Preparation of homogenate using Tissue Homogenizer

Figure 20: Homogenate sample

3.9.3.6.1 Catalase Test (CAT): The activity of catalase was evaluated utilizing a reaction solution that consisted of 2.5mL of 50mmol phosphate buffer (pH 5), 0.4mL of 5.9mmol H_2O_2 and 0.1mL of tissue homogenate. The changes in absorbance of the reaction solution at 240nm were recorded after a duration of one minute (Aebi Hugo, 1984). One unit of catalase activity is defined as absorbance change of 0.01 units per minute per mg of proteins.

$$\text{Catalase activity} = \{[\, 2.3 \times \log \frac{OD\ Initial}{OD\ Final}\ /\Delta t \times 100\ ] / 0.693\} / \text{mg of protein}$$

3.9.3.6.2 Glutathione Peroxidase assay (GPx): The glutathione peroxidase assay was performed by mixing 1.49mL of phosphate buffer (0.1mol; pH 7.4), 0.1mL of EDTA (1mmol), 0.05mL of glutathione reductase (1 IU/mL), 0.05mL of GSH (1mmol), 0.1mL of NADPH (0.2mmol), 0.01mL of H_2O_2 AND 0.1mL of homogenate. The reduction of NADPH at 340nm was recorded at 25° C. The enzyme activity was calculated as nmol of NADPH oxidized per minute per milligram of protein, utilizing a molar coefficient of 6.22 $\times 10^3\ M^{-1}\ cm^{-1}$ (Mohandas *et al*., 1984).

3.9.3.6.3 Superoxide Dismutase assay (SOD): The assessment of SOD activity was performed utilizing the method outlined by Kakkar *et al.*, 1984. The reaction mixture consisted of 0.1mL of phenazine methosulphate (186µmoL), 1.2mL of sodium pyrophosphate buffer (0.052mmol; pH 7.0) and 0.3mL of the supernatant of the homogenate. The enzymatic reaction was initiated with the addition of 0.2mL of NADH (780µmol) and terminated by the addition of 1mL of glacial acetic acid. The amount of chromogen produced was measured by evaluating the color intensity at 560nm. The results were expressed in units/mg of protein.

3.9.3.6.4 Lipid Peroxidation Assay (LPO): The TBARS test is used to determine the LPO assay in tissue homogenate. The test was performed using the method described by (Ottolenghi, 1959). In this assay, aliquots of the incubated samples were brought to a final volume of 2mL in centrifuge tubes. To each of these tubes, we added 1mL of 35% trichloroacetic acid, followed by 2mL of a 0.75% Thiobarbituric acid solution, making sure to mix thoroughly after each addition. The mixtures were then heated in a boiling waterbath for 15 minutes, with a bit of shaking. After cooling 2mL of 70% trichloroacetic acid was added. The tubes were mixed immediately again after 20 minutes to ensure the complete release of colored complex. Finally, the samples were centrifuged at 2500-3000 rpm for 15 minutes and measured the absorbance of the clear supernatant at 535 nm using a spectrophotometer.

Figure 21: Schimadzu UV-1900 UV-VIS Spectrophotometer

3.10 STATISTICAL ANALYSIS: Descriptive statistical analysis was depicted as mean ± SD (standard deviation). The data was analyzed using SPSS version 25 (Statistical Package for Social Science). To assess the significance of variance across the groups, one way analysis of variance was performed (One-way Anova) and *P value of less than 0.05 ($*p < 0.05$) was deemed to indicate significant differences among the experimental conditions.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 IDENTIFICATION OF SEAWEED: *Turbinaria decurrens* was identified as brown algae belong to the family *Sargassaceae* and was characterized by an upright thallus with radially branched axes having blades of tough texture. The blades were of turbinate forms and looks like a pinecone. This characteristic shape not only makes it visually recognizable but also contributes to its functional adaptation, helping it maximizes light capture while maintaining stability.

4.1.1 Authentication: The macroalgae was identified morphologically and taxonomically by a phycologist. A specimen along with herbarium (figure 22) was submitted to Sri Herbasia Biotech Private Ltd. Amritsar Punjab, India and a certificate (figure 23) with reference number SHB-22/23 -04 was obtained.

Figure 22: Herbarium of *Turbinaria decurrens* macroalgae collected from Gulf of Mannar coast Rameshwaram (Mandapam) India.

GSTIN : 03BFYPA1152D1ZX
Mfg. Lic No. 686-AY-PB

Sri Herbasia Biotech

A GMP & ISO 9001:2008 Certified Co.

Sri Herbasia Biotech
Care with Innovation
Serving Humanity with the Ayurveda

Ref. No. SHB-22/23-04

Dated 05-Nov-2022

PLANT AUTHENTICATION CERTIFICATE

The seaweed specimen which has been sent by you for authentication is identified as *Turbinaria decurrens Bory de Saint-Vincent;* Family- Sargassaceae. The seaweed specimen is returned herewith for preservation in your College/ Department/ Institution Herbarium.

To

Ms. Juhi Kataria
Ph.D. Research Scholar
Department of Medical Laboratory Sciences
School of Allied Medical Sciences
Lovely Professional University
Phagwara
Punjab

For Sri Herbasia Biotech

Prop.

ROHIT THAKKAR
M-Pharma (Ayu)

36, Lane No. 9, Industrial Area, Bal Kalan, Majitha Road, Amritsar. (Pb.) +91-9023069098, 9653538964
herbasiasales@gmail.com ; www.sriherbasiabiotech.com

Figure 23: Certificate of identification of seaweed *Turbinaria decurrens*

4.1.2 Phylogenetic analysis

4.1.2.1. Isolated genomic DNA: Genomic DNA was isolated from the seaweed samples by CTAB method as shown in figure 24. Approximately 25kb of genomic DNA was obtained. DNA concentration results indicated that 73.7µg/sample have a purity of 1.87 (Table 1).

Table 1: Quantification of DNA

Sample Code	Concentration (µg/mL)	Purity (A260/A280)
Sample	73.7	1.87

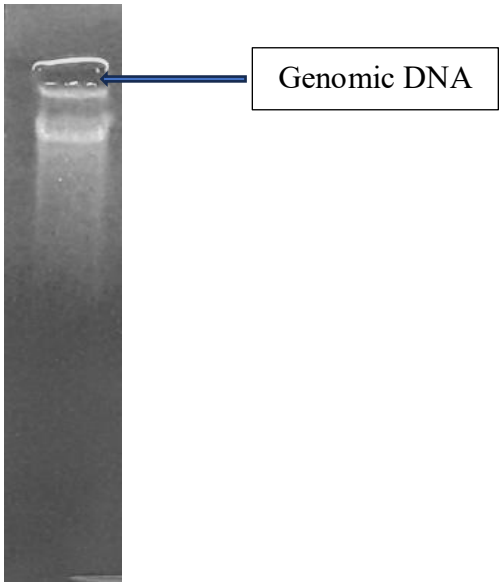


Figure 24: Genomic DNA of selected seaweed sample

4.1.2.2. Amplification and electrophoresis: The extracted genomic DNA was subjected to the amplification of the *rbcL* gene in 1.5% agarose gel electrophoresis (figure 25). The expected amplicon size was nearly 757bp and also been recorded the same size in the obtained amplicon. The amplified product was purified using the Exo-sap method and run in an ABI Prism gene sequencer. About 757 base pairs were obtained in the sequence through forward primer.

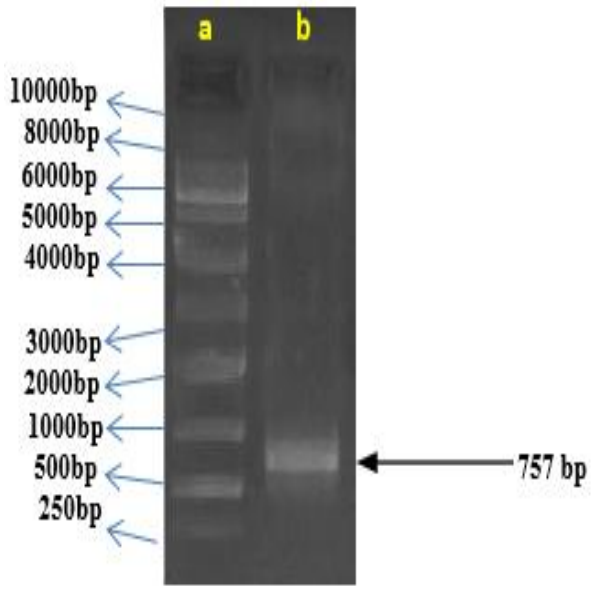


Figure 25: PCR amplification profile of selected plants sample

Conditions: 1.5% agarose gel electrophoresis

(Lane a: 1kb DNA Ladder; b: Sample)

1 KB DNA Ladder (bp):10000, 8000, 6000, 5000, 4000, 3000, 2000, 1000, 500, 250

4.1.2.3. Sequencing results for *Turbinaria decurrens*: The sequences analyzed BLAST program at the NCBI website showed up to 99.3% sequences similarity with existing plant *rbcL* gene sequences, specifically with Genbank accession number DQ448830.1.

***Turbinaria decurrens* (# DQ448830)**

TGGCAATTTGGGCTCGAAAAGCTGAGATGATTTTACATTTACATCGTGCTGGTAATTCTACTTATGCACGTCAAAAAAAT
CATGGAATTAACTTTAGGGTTATTTGTAAATGGATGCGTATGTCCGGTGTAGATCATATTCATGCAGGTACAGTTGTGGG

GAAACTAGAAGGAGATCCTTTGATGGTTAGAGGATTTTACAATACGCTATTGTTAACTGAATTAAAAATTAATCTGGCTG

AAGGAATATTCTTCGATATGGATTGGGCATCGCTTAGAAAATGTGTTCCTGTAGCATCAGGTGGTATTCATTGTGGTCAA

ATGCATCAACTTCTTTATTATTTAGGTGATGATGTTGTTCTACAATTTGGAGGTGGTACAATTGGTCACCCTGATGGTAT

ACAAGCTGGTGCTACAGCAAATCGTGTTGCTTTAGAAGCAATGGTTCTAGCTAGAAATGAGGGTCGTGATTATGTTGGTG

AAGGGCCAGAAATTTTACGTACTGCTGCAAGTACTTGTGGACCTTTAAAAGCAGCTTTAGATCTATGGAAAGATATTACT

TTTGAATATACTTCAACAGACACACCTGATTTTGTTGAAGTAGCAACTGAAAGTCCATAAAGTTATTCTGTTCTACAGTT

TAATTTTTATTATAAAATCTAAAAGAATATATTAGTGAGCTTTAATCTTTTTAATACTTTACATTAAAATAAAAAACATA

AAAAGTTTGGTAGTTAACTAAAAATAAAACTAAAATATTTA

Turbinaria conoides (# DQ448833)

TGGCAATTTGGGCTCGAAAAGCTGAGATGATTTTACATTTACATCGTGCTGGTAATTCTACTTATGCACGTCAAAAAAAT

CATGGGATTAACTTTAGGGTTATTTGTAAATGGATGCGTATGTCCGGTGTAGATCATATTCACGCGGGTACAGTTGTGGG

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AAGGGATATTCTTCGATATGGATTGGGCATCGCTGAGAAAATGTGTTCCTGTAGCATCAGGTGGTATTCATTGTGGTCAA

ATGCATCAACTTCTTTATTATTTAGGTGATGATGTTGTTCTACAATTTGGAGGTGGTACAATTGGTCACCCTGATGGTAT

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AAGGACCAGAAATTTTACGTACTGCTGCAAGTACTTGTGGACCTTTAAAAGCAGCTTTAGATCTATGGAAAGATATTACT

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TAATTTTTATTATAAAATCTAAAAGAATATATTAGCGAGCTTTAATCTTTTTAATACTTTACATTAAAATAAAAAACATA

AAAAGTTTGGTAGTTAACTAAAAATAAAACTAAAATATTTA

Turbinaria ornata (# KY432511)

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AAAAGTTTGGTAGTTAACTAAAAATAAAACTAAAATATTTA

Turbinaria foliosa (# EU169863)

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CATGGTATTAACTTTAGGGTTATTTGCAAATGGATGCGTATGTCCGGTGTAGATCATATTCACGCTGGTACAGTTGTAGG

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AAAAGTTTGGTAGTTAACTAAAAATAAAATTAAAATATTTA

Turbinaria turbinata (# EU169862)

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TAATTTTTACTATAAAAGAATTTATTAGTGAACTTTAATCTTTTTAATATTTTACATTAAAACAAAAAACATAAAAAGTT

TGGTAGTTAACTAAAAATAAAACTAATAATATTTA

Turbinaria gracilis (# JN243839)

GGGATTAACTTTAGGGTTATTTGTAAATGGATGCGTATGTCCGGTGTAGATCATATTCACGCGGGTACAGTTGTGGGGAA

ACTAGAAGGAGATCCTTTGATGGTTAGAGGATTTTACAATACGCTATTGTTAACTGAATTAAAAATTAATCTAGCTGAAG

GGATATTCTTCGATATGGATTGGGCATCGCTGAGAAAATGTGTTCCTGTAGCATCAGGTGGTATTCATTGTGGTCAAATG

CATCAACTTCTTTATTATTTAGGTGATGATGTTGTTCTACAATTTGGAGGTGGTACAATTGGTCACCCTGATGGTATACA

AGCCGGTGCTACAGCAAATCGTGTTGCTTTAGAAGCAATGGTTCTAGCTAGAAATGAGGGTCGTGATTATGTTGGTGAAG

GACCAGAAATTTTACGTACTGCTGCAAGTACTTGTGGACCTTTAAAAGCAGCTTTAGATCTATGGAAAGATATTACTTTT

GAATATACTTCAACAGACACACCTGATTTTGTTGAAGTAGCAACTGAAAGTCCATAAAGTTATTCTGTTCTACAGTTTAA

TTTTTATTATAAAATCTAAAAGAATATATTAGCGAGCTTTAATCTTTTTAATACTTTACATTAAAATAAAAAACATAAAA

AGTTTGGTAGTTAACTAAAAATAAAACTAAAATATTTA

Myagropsis myagroides (# KY935450)

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Sargassum fulvellum (# NC_066457)

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TGATTTTTATTATAAAATCTAAAAGAACAGATTAATGTACGTTAATCTTTTTAATATTTCACATTAAAATAAAAGACATA

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Coccophora langsdorfii (# NC_032288)

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GAAACTAGAAGGGGATCCTTTGATGGTTAGAGGATTTTACAATACATTATTGTTAACTGAATTAAAAATTAATTTAGCTG

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TTAATTTTCACTATAAAATATAAAAGAATAGATCAATGAACTTTGATCTTTTTAATATTTTACATTAATAATAAAGAAGA

TAAAAAGTTTGGTAGTTAACTAAAAATAAAATTAAAATATTTA

 # Seaweed Sample

TGGCAATTTGGGCTCGAAAAGTGAGATGATTTTACATTTACATCGTGCTGGTAATTCTACTTATGCACGTCAAAAAAATC

ATGGAATTAACTTTAGGGTTATTTGTAAATGGATGCGTATGTCCGGTGTAGATCATATTCATGCAGGTACAGTTGTGGGG

AAACTAGAAGGAGATCCTTTGATGGTTAGAGGATTTTACAATACGCTATTGTTAACTGAATTAAAAATTAATCTGGCTGA

AGGAATATTCTTCGATATCGATTGGGCATCGCTTAGAAAATGTGTTCCTGTAGCATCAGGTGGTATTCATTGTGGTCAAA

TGCATCAACTTCTTTATTATTTAGGTGATGATGTTGTTCTACAATTTGGAGGTGGTACAATTGGTCACCCTGATGGTATA

CAAGCTGGTGCTACAGAAATCGTGTTGCTTTAGAAGCATGGTTCTAGCTAGAAATGAGGGTCGTGAATATGTTGGTGAAG

GGCCAGAAATTTTACGTACTGCTGCAAGTACTTGTGGACCTTTAAAAGCAGCTTTAGATCTATGGAAAGATATTACTTTT

GAATATACTTCAACAGACACACCTGATTTTGTTGAAGTAGCAACTGAAAGTCCATAAAGTTATTCTGTTCTACAGTTTAA

TTTTTATTATAAAATCTAAAAGAATATATTAGTGAGCTTTAATCTTTTTAATACTTTACATTAAAATAAAAAACATAAAA

GTTTGGTAGTTAACTAAAAATAAAACTAAAATATTTA

4.1.2.4. Phylogenetic analysis: The phylogenetic analysis of seaweed sample was constructed based on their *rbcL* gene sequences and alignment using CLUSTAL W program. The aligned sequences were further analysed utilizing Maximum Parsimony method to construct phylogenetic tree of the seaweed sample. The resulting phylogenetic tree is illustrated in figure 26. The tree shows the relationships among various *Turbinaria* species and their closest relatives within the brown algae group. It also included other algal species such as *Myagropsis* and *Sargassum*, highlighting the evolutionary links between these species. The optimal tree with the sum of branch length = 0.46500602 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) were shown next to the branches (Felsenstein, 1985). Bootstrap analysis was done based on 1000 replications.

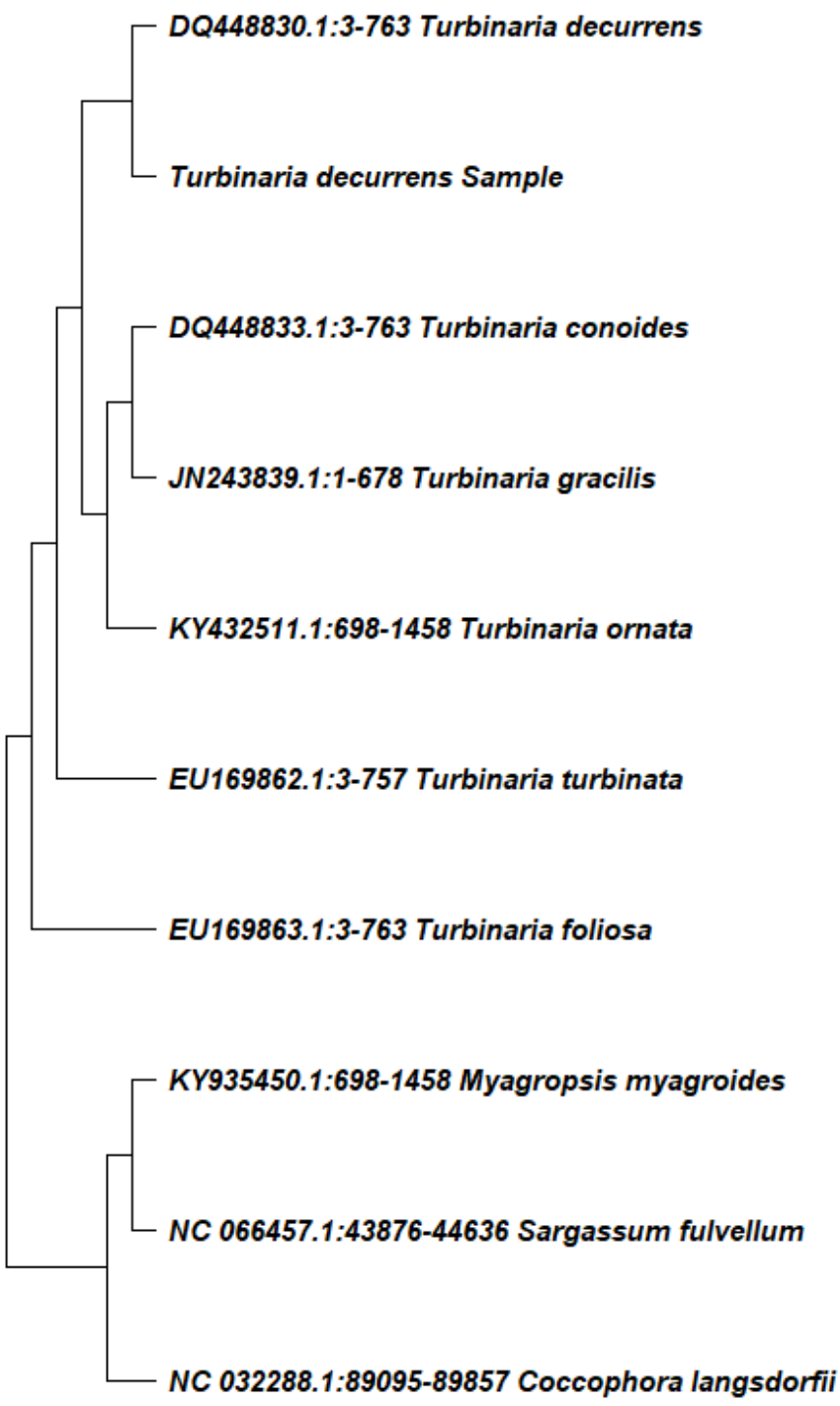


Figure 26: Phylogenetic tree of Family *Sargassaceae* and related sequences

4.2. SOXHLET EXTRACTION & TOTAL YIELD OF *TURBINARIA DECURRENS*:

4.2.1. Dry yield of *Turbinaria decurrens:* The weight of seaweed was measured using a weighing balance. The samples of seaweeds were collected from three different locations weighing 1000grams, 950grams and 900grams. The wet weight was measured, and it was incubated at 37°C for 24 hours to obtain dry yield. The dry yield obtained was 79grams, 74grams and 69grams. The percent yield obtained was 7.9%, 7.4% and 6.9% (Table 2).

Table 2: Dry yield of *Turbinaria decurrens*

Wet weight (grams)	Period of drying	Dry weight (grams)	Yield percentage (%)
1000	Incubation at 37°C for 24 hours	79±0.3	7.9
950		74±0.4	7.4
900		69±0.7	6.9

Values expressed as Mean ± SD

4.2.2 Total yield on solvent extraction of *Turbinaria decurrens*: Seaweed samples subjected to Soxhlet extraction to obtain the bioactive compounds, used three different solvents *viz* acetone, methanol and aqueous which yielded 6.32%, 7.64% and 6.58% respectively (Table 3). The total yield obtained from methanol extract was greater as compared to the aqueous and acetone extracts.

Table 3: Percentage yield of *Turbinaria decurrens* using different solvents

Sample Quantity (Grams)	Solvent	Yield (grams)	Results (Yield%)
25	Acetone	1.58±0.10	6.32
25	Aqueous	1.64±0.05	6.58
25	Methanol	1.91±0.08	7.64

Values expressed as Mean ± SD

The findings of this study explored the efficiency of three different solvents acetone, methanol and water in extracting bioactive constituents from seaweed via Soxhlet extraction. Among these, methanol demonstrated the highest extraction yield (7.64%), followed by water and acetone. This variation in yield can be attributed to the polarity and solvation power of the solvents used. Methanol's superior extraction performance is likely due to its intermediate polarity, allowing it to dissolve a broader range of polar and semi-polar phytochemicals. It is well established that methanol is highly effective insolubilizing phenolics, flavonoids and other bioactive constituents typically present in seaweeds (Hasan *et al*., 2022). The relative difference observed in extraction yield were probably due to differences in solvent polarity and their interaction with bioactive compounds. Though acetone is commonly employed solvent in plant extraction but in this research findings, less percentage of yield (6.32%) was obtained as compared to methanol. Consequently, methanol can be regarded as an appropriate solvent for recovery of biologically active components in seaweed extraction methodologies.

4.3. PRELIMINARY PHYTOCHEMICAL ASSESSMENT: The dried yield was analyzed qualitatively and quantitatively to assess the bioactive compounds present in the seaweed sample.

4.3.1. Qualitative observations: In the phytochemical qualitative analysis of seaweed extracts, it was observed that valuable secondary metabolites such as coumarins, glycosides, phenol, tannins and terpenoids were present in the crude extract derived from all the three solvents (Table 4), in agreements with earlier studies that have identified genus *Turbinaria* promising anti-obesity, antioxidant, cardioprotective, anti-microbial and antiviral properties of marine algae (Rushdi *et al*., 2021b).

Recent studies on marine macroalgae have identified gallic acid, a phenolic compound with notable cytotoxic effects against H460 and MCF-7 cell lines, highlighting the therapeutic promise of *Turbinaria decurrens* (Sami & Nur, 2022). In another investigation cyclotrisiloxane was detected, a bioactive molecule potentially contributing to the seaweed's antioxidant and antidiabetic properties (Ismail *et al*., 2020). Furthermore, the presence of glycosides, tannins (Yu *et al*., 2021) and terpenoids in all three extracts of

Turbinaria decurrens further enriches its phytochemical profile (figure 27), as these secondary metabolites are well-recognized for their roles in plant defense, antioxidative mechanisms and bioactivity modulation through pathways such as enzyme inhibition and radical scavenging.

Table 4: Results of phytochemical analysis of *Turbinaria decurrens*

S. No.	Components	Acetone extract	Aqueous extract	Methanol extract
1.	Alkaloids	Absent	Absent	Absent
2.	Anthraquinones	Absent	Absent	Absent
3.	Coumarins	**Present**	**Present**	**Present**
4.	Glycosides	**Present**	**Present**	**Present**
5.	Flavonoids	Absent	Absent	Absent
6.	Phenol	**Present**	**Present**	**Present**
7.	Saponins	Absent	Absent	Absent
8.	Steroids	Absent	Absent	Absent
9.	Tannins	**Present**	**Present**	**Present**
10.	Terpenoids	**Present**	**Present**	**Present**
Total phytochemicals present		05	05	05

a) Acetone extract

b) Aqueous extract

c) Methanol extract

Figure 27: Qualitative estimation of Phytochemical constituents of *Turbinaria decurrens*

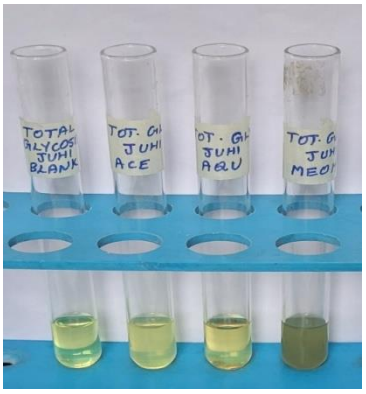
4.3.2. Quantitative observations: The seaweed derived elements identified via qualitative analysis were subjected to additional quantification to ascertain their concentrations (Table 5).

Table 5: Results of Quantitative phytochemical analysis of bioactive compounds present in the crude extracts of *Turbinaria decurrens*

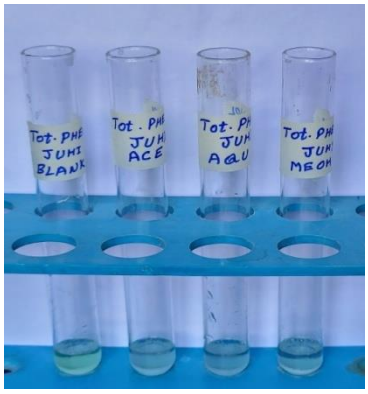
S. NO.	ASSAY	UNIT	RESULTS		
			Acetone Extract	Aqueous Extract	Methanol Extract
1	Total Coumarin Content	mg Esculin equivalent/g DE	124.9±0.95	101.8±0.41	161.21±0.36
2	Total Glycosides Content	mg digoxin equivalent/g DE	66.81±0.18	29.92±0.15	366.87±0.06
3	Total Phenol Content	mg Gallic acid equivalent/g DE	225.06±0.68	206.71±0.67	242.26±0.61
4	Total Tannin Content	mg Gallic acid equivalent/g DE	194±0.59	181.54±0.26	204.89±0.54
5	Total Terpenoid Content	mg Linalool equivalent/g DE	294.08±0.33	181.7±0.69	393.78±0.11

Values expressed as Mean ± SD

a) Total Coumarin Content

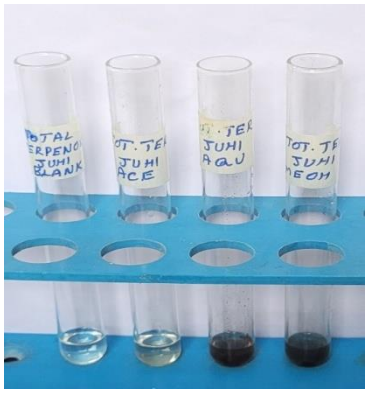


b) Total Glycoside Content



c) Total Phenol Content

d) Total Tannin Content



e) Total Terpenoid Content

Figure 28: Quantitative estimation of Phytochemical constituents of *Turbinaria decurrens*

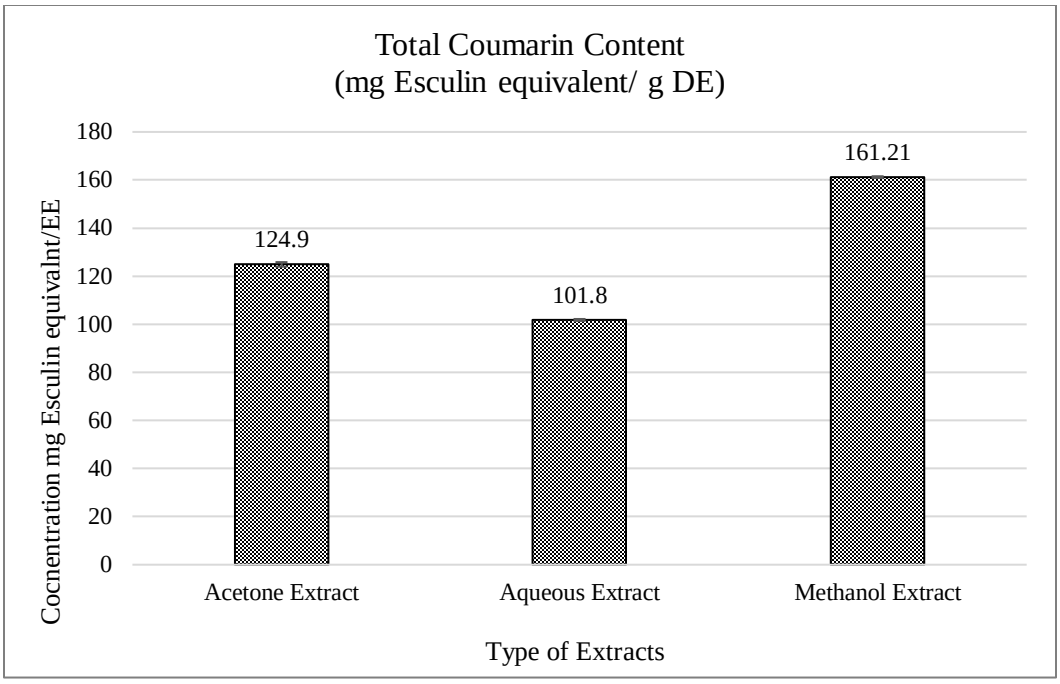


Figure 29: Total Coumarin Content (mg Esculin equivalent/ g DE) in crude extract

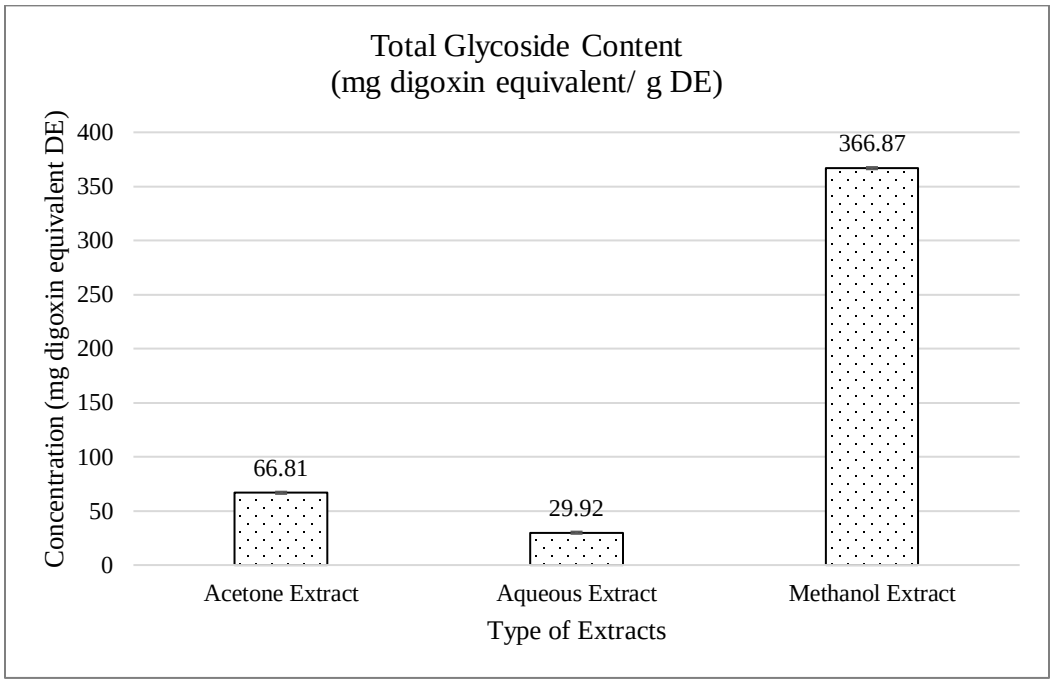


Figure 30: Total Glycoside Content (mg digoxin equivalent/ g DE) in crude extracts

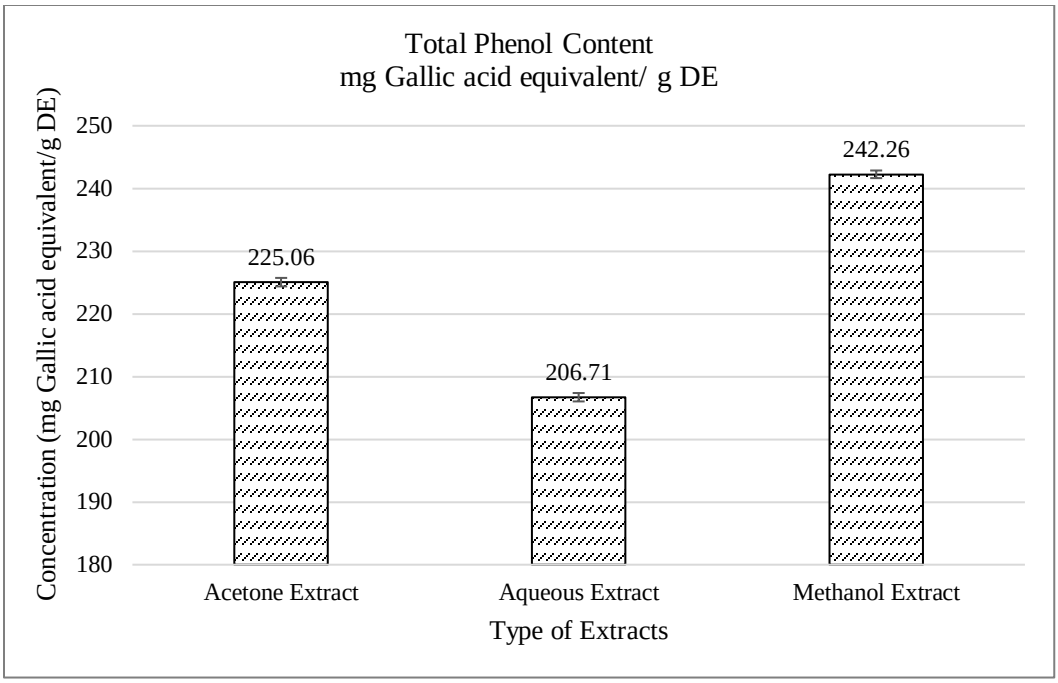


Figure 31: Total Phenolic Content (mg Gallic acid equivalent/g DE) in crude extracts

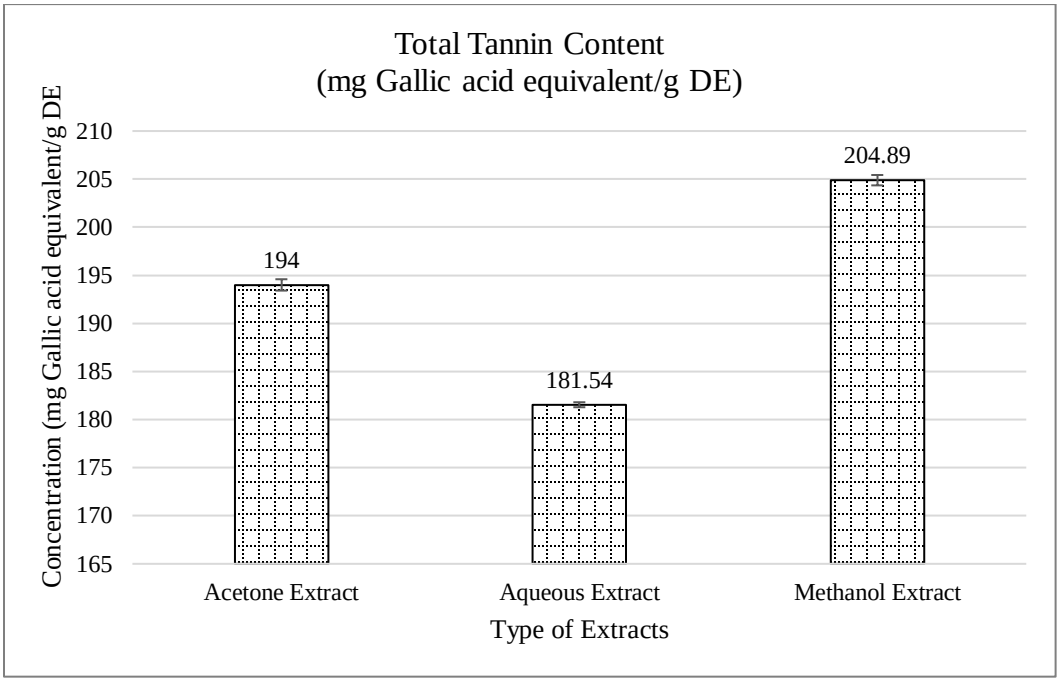


Figure 32: Total Tannin Content (mg Gallic acid equivalent/g DE) in crude extracts

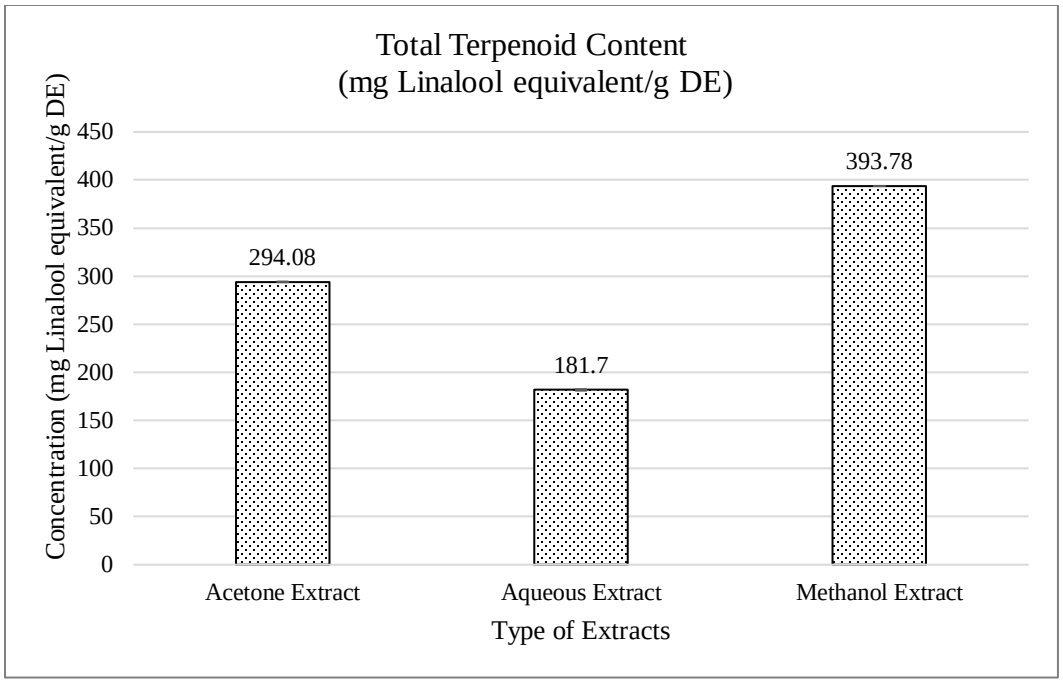


Figure 33: Total Terpenoid Content (mg Linalool equivalent/g DE) in crude extracts

4.3.2.1 Total Coumarin Content: Total coumarin content was expressed as mg Esculin equivalent/DE. The quantity of coumarin content was highest from the *Turbinaria decurrens* methanolic extract (figure 29) (161.21±0.36mg esculin equivalent/ g dry extract) followed by acetone (124.9±0.95mg esculin equivalent/ g dry extract) and aqueous extract (101.8±0.41 mg esculin equivalent/ g dry extract).

4.3.2.2 Total Glycoside Content: Total glycoside was expressed in mg digoxin equivalent/ g dry extract. The methanolic extract showed an increased amount (366.87±0.06mg digoxin equivalent/g dry extract) of total glycosides while acetone (66.81±0.18mg digoxin equivalent/g dry extract) and aqueous extract (29.92±0.15mg digoxin equivalent/g dry extract) showed lower quantities (figure 30).

4.3.2.3 Total Phenolic Content: The total phenolic content was expressed as gallic acid equivalents (GAE) in milligram per gram of dry extract (mg GAE/g dry extract). The total phenolic compound recovered from the *Turbinaria decurrens* methanolic extract was 242.26±0.61mg GAE/g dry extract, whereas the amount retrieved from the aqueous extract and acetone extract was 225.06±0.68 mg GAE/g dry extract and 206.71±0.67 mg GAE/g

dry extract respectively (figure 31). According to Arguelles *et al*., 2020 the methanolic extract of *Turbinaria decurrens* exhibited a total phenolic content 27.84±0.12 mg GAE/g. In a related study, *Turbinaria conoides* and *Turbinaria ornata* ethyl acetate fractions showed considerably higher phenolic levels at 105.97 mg GAE/g and 69.63 mgGAE/g respectively (Chakraborty *et al*., 2015). The present findings are consistent with previous findings as methanol proved to be the most effective solvent for phenolic extraction. Owing to its strong polarity, methanol facilitates efficient solubilization of phenolic and other polar bioactive compounds. Recent investigation has demonstrated the methanolic extracts of various brown seaweeds, including *Ecklonia* and *Fucus species* yielded substantial phenolic content (Duan *et al*., 2023).

4.3.2.4 Total Tannin Content: The total tannin content was expressed as gallic acid equivalents (GAE) in milligram per gram of dry extract (mg GAE/g dry extract). The quantity of tannin content in methanolic extract was 204.89±0.54mg GAE/g dry extract, acetone and aqueous extract has 194±0.59mg GAE/g dry extract and 181.54±0.26mg GAE/g dry extract respectively (figure 32). The total tannin content in methanol extract was elevated as compared to acetone and aqueous extract. It's a type of natural active substance extracted, having biological activities such as antioxidant antibacterial, anti-viral, anti-tumour, anti-hypertensive and hypoglycemic (Zheng *et al*., 2022). Although quantitative tannin data specific to *Turbinaria decurrens* remains limited, general phytochemical investigations in brown algae including other *Turbinaria* species consistently affirm the presence of bioactive significance of tannins.

4.3.2.5 Total Terpenoid Content: Total terpenoid content was expressed as mg Linalool equivalent/g dry extract. The total content of terpenoid was higher in methanol extract (393.78±0.11mg Linalool equivalent/g dry extract) followed by acetone extract (294.08±0.33mg Linalool equivalent/g dry extract) and aqueous extract has relatively lesser quantities of 3 (figure 33) (181.7±0.69mg Linalool equivalent/g dry extract).

The elevated terpenoid content observed in methanol extract of *Turbinaria decurrens* is particularly noteworthy, as terpenoids constitute a diverse class of secondary metabolites known for their wide range of biological activities. These compounds have been

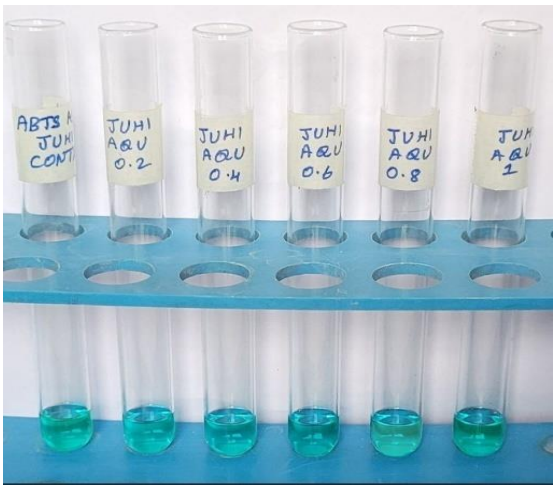
documented for their anti-inflammatory and anticancer properties, and are often implicated in the chemical defense strategies of marine algae (Chen *et al*., 2023).

Variations in the solvent performance for phytochemical extraction indicate that the choice of solvent plays a crucial role in maximizing yields of specific bioactive compounds. In this study, methanol facilitated superior recovery of certain metabolites from *Turbinaria decurrens,* demonstrating a strong correlation between its rich phytochemical composition and enhanced biological activities, highlighting its superiority over acetone and aqueous extract. Quantitative phytochemical analysis revealed that methanolic extract contained the higher concentrations of total phenolics, tannins, terpenoids and coumarins. Methanol's intermediate polarity efficiently solubilizes both polar and semi-polar secondary metabolites is responsible for the increased recovery of these bioactive constituents. These compounds are well recognized for their redox potential and membrane protective properties, thereby providing a biochemical basis for the pronounced biological activities observed.

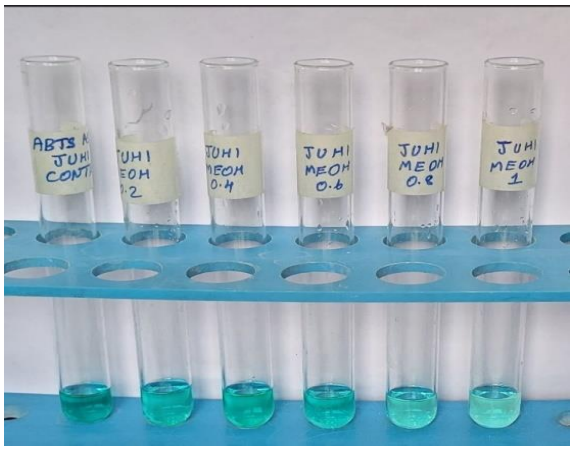
4.4. ANTIOXIDANT ACTIVITY: The antioxidant activity was evaluated using ABTS, DPPH, FRAP & LPO. These results provide insight into the free radical scavenging ability and overall antioxidant potential of the extracts.

4.4.1. 2,2 azino bis (3-ethylbenzthiazoline)-6-sulfonic acid assay (ABTS assay) – The results showed that among the three extract studied, methanolic extract of *Turbinaria decurrens* had the highest percentage 28.02±15.2% of scavenging activity with IC_{50} value 1.06mg/mL for all the concentrations (0.2-1.0mg/mL) followed by the aqueous extract (18.75±8.93%) with IC_{50} of 1.62mg/mL and, acetone extract (17.67±8.29%) with IC_{50} of 1.75mg/mL (Table 6). These findings indicated that the methanolic extract of *Turbinaria decurrens* exhibited potent scavenging activity ($p<0.05$) that can be compared with ascorbic acid.

a) Acetone extract



b) Aqueous extract



c) Methanolic extract

Figure 34: ABTS assay of all three extracts of *Turbinaria decurrens* (concentration 0.2-1mg/mL)

Table 6: *In-vitro* ABTS antioxidant activity of Acetone, aqueous and methanolic extracts of *Turbinaria decurrens*

Concentration (mg/mL)	Acetone (% inhibition)	Aqueous (% inhibition)	Methanolic (% inhibition)
0.2	6.59±0.22	6.69±0.38	11.43±0.22*
0.4	14.13±0.36	13.99±0.36	16.36±0.14*
0.6	16.69±0.16	18.21±0.18	26.08±0.22*
0.8	22.05±0.14	25.65±0.22	39.26±0.28*
1	28.17±0.38	29.16±0.21	47.8±0.14*
IC_{50}	1.75	1.62	1.06*

Values expressed as Mean ± SD. Statistical analysis was performed using one-way analysis of variance with post-hoc testing. *= p<0.05

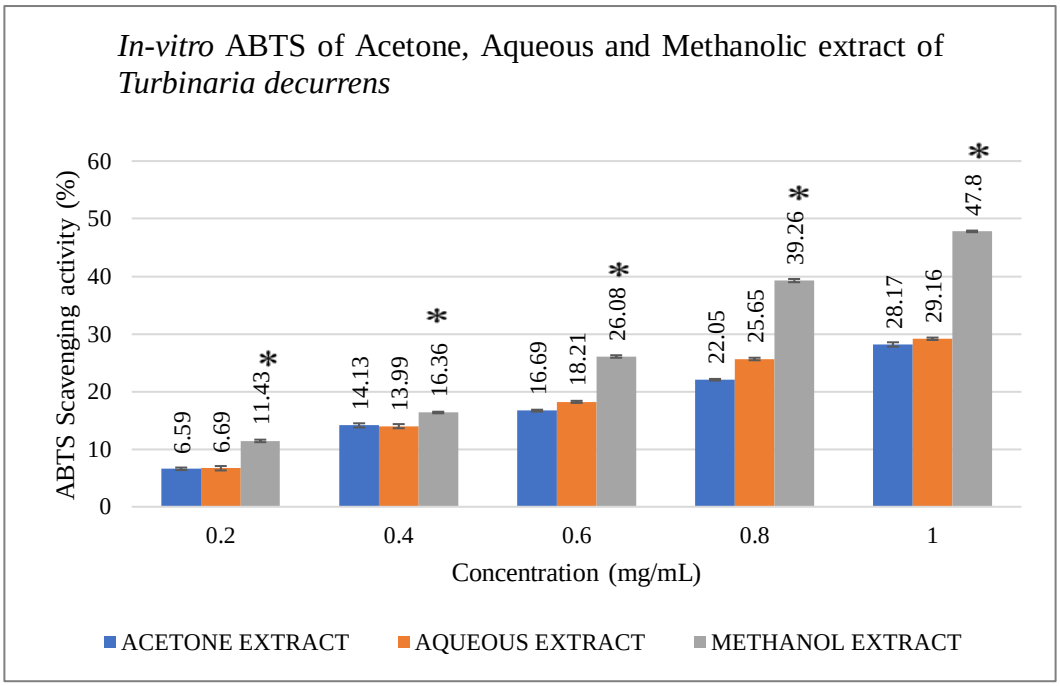


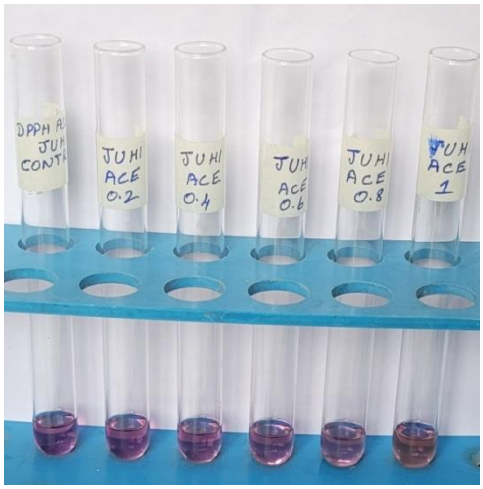
Figure 35: Graphical representation of ABTS antioxidant activity using Acetone, aqueous and methanolic extract of *Turbinaria decurrens.* Statistical analysis was performed using one-way analysis of variance with post-hoc testing. *= p<0.05

Table 7: ABTS Antioxidant activity one way ANOVA

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1383.891	4	345.9727	7.222435	0.0053	3.47805
Within Groups	479.0249	10	47.90249			
Total	1862.916	14				

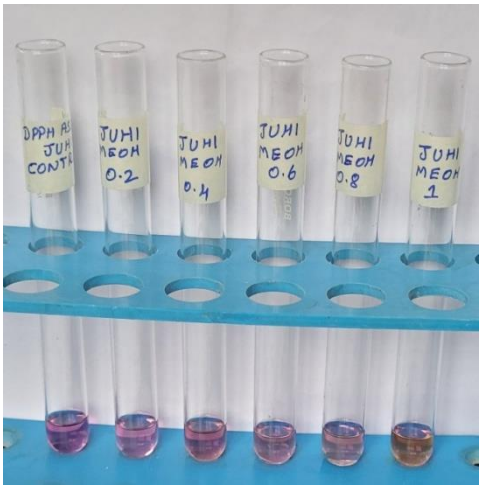
The different concentrations of ABTS used in the experiment likely influenced the amount of ABTS•+ generated. In the present investigation, *Turbinaria decurrens* displayed strong ABTS radical scavenging activity, which can be attributed to its abundant phenolic content. Similarly, the aqueous extract of *Turbinaria ornata* showed a concentration dependent reduction of ABTS•+ radicals, with potency of surpassing reference antioxidant Trolox (Ananthi *et al*., 2011). Another comparative study had shown that the methanolic extract of *Turbinaria decurrens* demonstrated strong ABTS radical scavenging capacity, which is consistent with its performance in other antioxidant assays (Arguelles *et al*., 2020). Comparable study reported that the ethyl acetate fraction of brown seaweed *Sargassum wightii* (Kumar *et al*., 2022) also displayed notable antioxidant potential, further supporting the role of phenolics in the antioxidant activity of brown seaweeds.

4.4.2. 1, 1-diphenyl-2-picrilhydrazyl assay (DPPH assay): In the present study, DPPH radical scavenging activity was evaluated using concentrations of 0.2, 0.4, 0.6, 0.8, and 1mg/mL. The methanolic extract of *Turbinaria decurrens* showed the highest antioxidant activity among the tested solvents (Table 8).



a) Acetone extract

b) Aqueous extract



c) Methanol extract

Figure 36: DPPH assay of all three extracts of *Turbinaria decurrens* (concentration 0.2-1mg/mL)

Table 8: *In-vitro* DPPH antioxidant activity of acetone, aqueous and methanolic extracts of *Turbinaria decurrens*

Concentration (mg/mL)	Acetone (% inhibition)	Aqueous (% inhibition)	Methanol (% inhibition)
0.2	3.7±0.16	7.78±0.24	9.02±0.16*
0.4	8.91±0.34	13.85±0.16	15.62±0.16*
0.6	12.18±0.25	18.95±0.25	19.16±0.11*
0.8	15.73±0.34	21.69±0.25	23.19±0.16*
1	19±0.43	27.05±0.32	32.15±0.24*
IC_{50}	2.54	1.75	1.56*

Values expressed as Mean ± SD. Statistical analysis was performed using one-way analysis of variance with post-hoc testing. *= p<0.05.

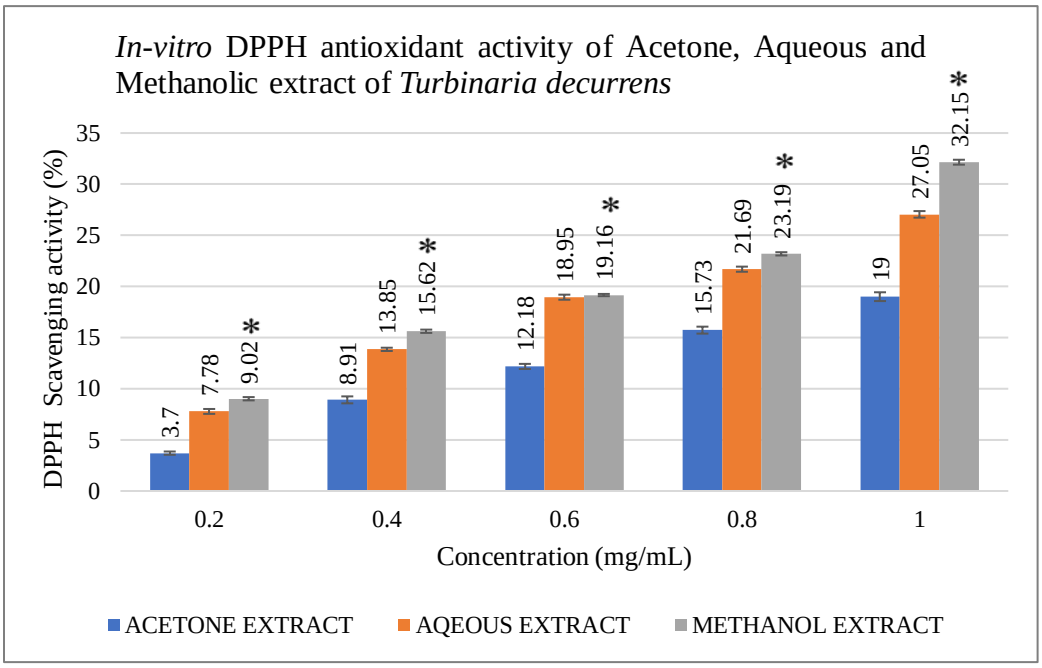


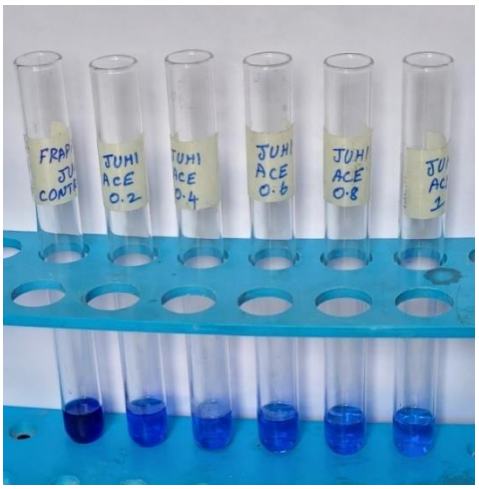
Figure 37: Graphical representation of DPPH antioxidant activity using Acetone, aqueous and methanolic extract of *Turbinaria decurrens.* Statistical analysis was performed using one-way analysis of variance with post-hoc testing. *= p<0.05

Table 9: DPPH Antioxidant activity one way ANOVA

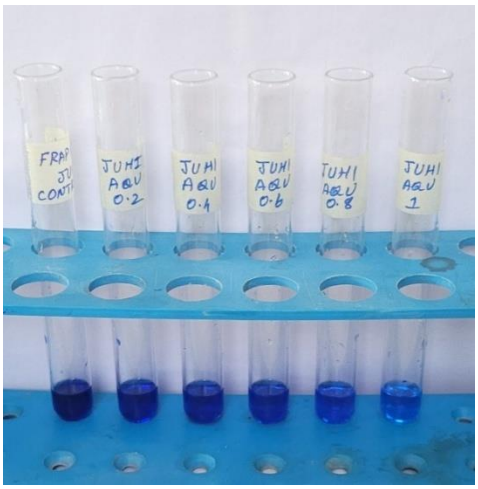
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	637.4515	4	159.3629	8.375726	0.003113	3.47805
Within Groups	190.2675	10	19.02675			
Total	827.719	14				

The percentage scavenging activity of methanolic extract was 19.81±8.70% and an IC_{50} value of 1.56mg/mL. The aqueous extract followed closely exhibited 17.84±7.55 percent inhibition and an IC_{50} 1.75mg/mL. In contrast the acetone extract showed the lowest activity with an inhibition rate of 11.88±6.20% and IC_{50} 2.54mg/mL. Statistical analysis revealed a significant difference ($p < 0.05$) in DPPH activity between the different solvent extracts (figure 37). An increase in the extract concentration was associated with greater capacity for DPPH scavenging. These results align with the findings of Kalimuthu & Shankar, 2021 who reported strong antioxidant activity of *Turbinaria decurrens* reaching 92.3% inhibition at 100µg/mL. Additionally, previous research evaluating antioxidant capacity in various seaweeds *viz Turbinaria decurrens, Padina pavonica, Sargassum muticum, Ulva lactuca and Pterocladia capillacea* confirmed their antioxidant potential and among them acetone extract of *Turbinaria decurrens* showed highest antioxidant activity which is aligned with our findings (Ismail *et al*., 2020).

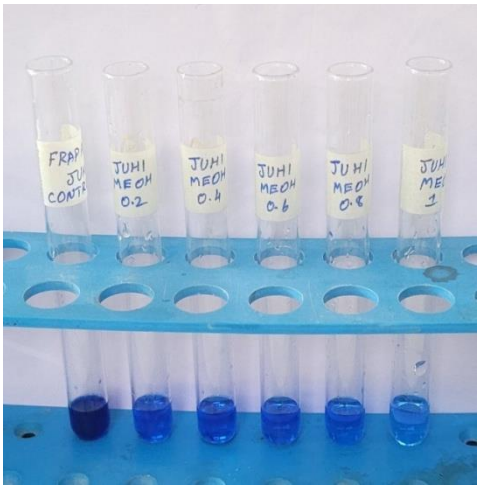
4.4.3. Ferric reducing antioxidant power assay (FRAP): FRAP concentrations of 0.2, 0.4, 0.6, 0.8, and 1mg/mL were used in the study.



a) Acetone extract



b) Aqueous extract



c) Methanolic extract

Figure 38: FRAP assay of all three extracts of *Turbinaria decurrens* (concentration 0.2-1mg/mL)

Table 10: *In-vitro* FRAP assay of acetone, aqueous and methanolic extracts of *Turbinaria decurrens*

Concentration (mg/mL)	Acetone (% inhibition)	Aqueous (% inhibition)	Methanol (% inhibition)
0.2	35.15±0.12	36.4±0.18	37.81±0.29*
0.4	39.15±0.18	38.45±0.19	44.02±0.18*
0.6	43.35±0.12	42.23±0.24	46.44±0.11*
0.8	46.2±0.31	48.4±0.11	50.91±0.24*
1	50.99±0.25	52.73±0.36	55.23±0.31*
IC_{50}	0.96	1.50	0.74*

Values expressed as Mean ± SD. Statistical analysis was performed using one-way analysis of variance with post-hoc testing. *= p<0.05

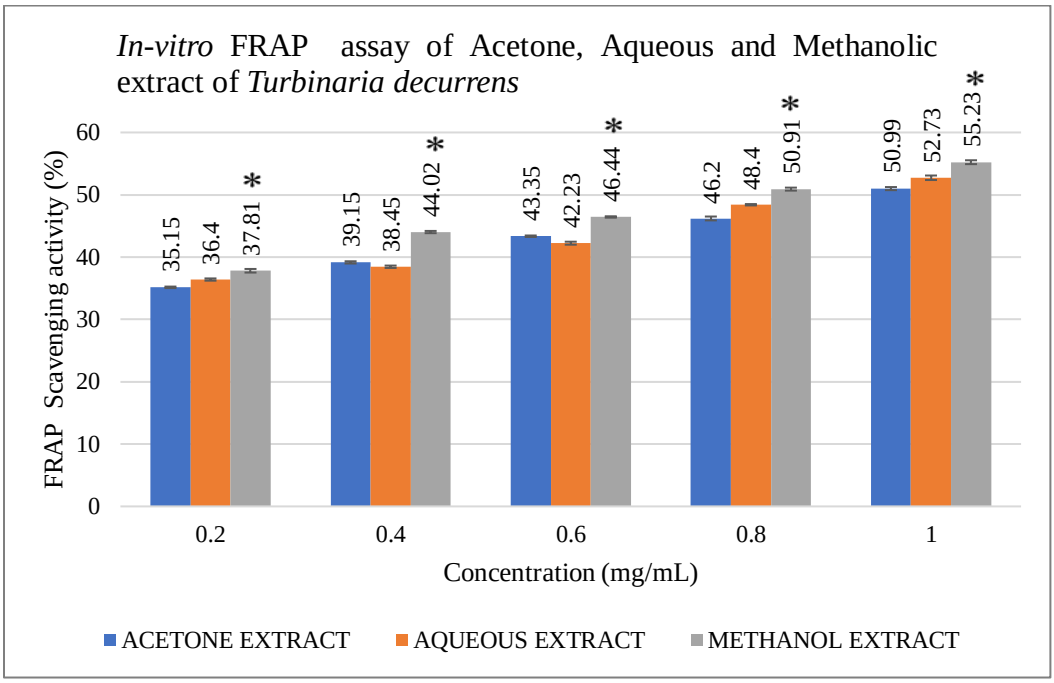


Figure 39: Graphical representation of FRAP assay using Acetone, aqueous and methanolic extract of *Turbinaria decurrens*. Statistical analysis was performed using one-way analysis of variance with post-hoc testing. *= p<0.05

Table 11: FRAP assay One way ANOVA

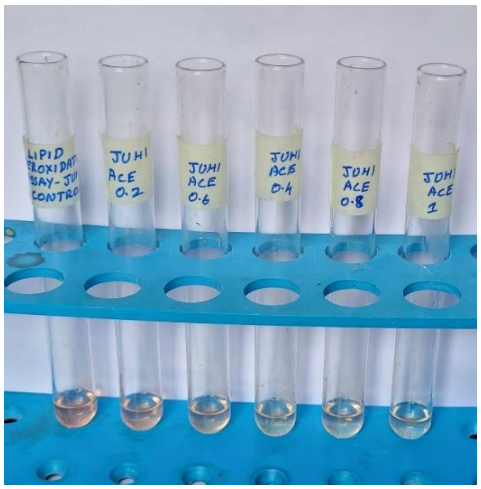
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	635.2036	4	158.8009	1.075653	0.41839	3.47805
Within Groups	1476.321	10	147.6321			
Total	2111.525	14				

The methanolic extract of *Turbinaria decurrens* demonstrated the highest antioxidant activity, showing percentage inhibition (%) of 46.84±6.74 and an IC_{50} value of 0.74mg/mL for FRAP assay (Table 10). This was followed by the acetone extract which exhibited slightly lower antioxidant potential with an inhibition rate of 42.99±6.22% and an IC_{50} 0.96mg/mL. The aqueous extract showed the least activity recording 24.49±9.45% inhibition and an IC_{50} of 1.50mg/mL.

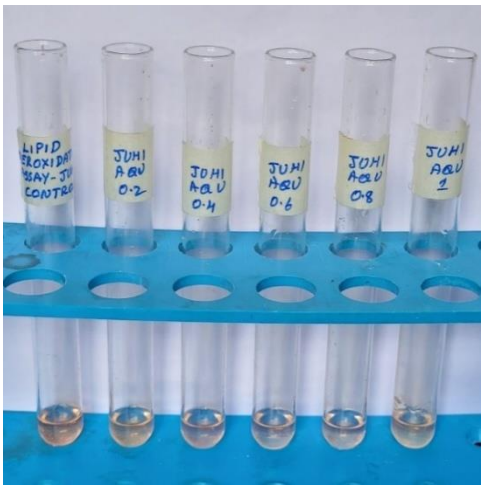
In a comparative analysis of six brown macroalgal species namely *S. vestitum, S linearifolium, P. comosa, Padina Sp., H. banksia and S. podocanthum* significant differences in antioxidant activity were found. The brown seaweed *S. vestitum* especially showed a higher FRAP activity due to the presence of bioactive phytochemical in it (Dang *et al*., 2018). In addition to this, FRAP IC_{50} values for different *Turbinaria ornata* extracts were found to be 41.99 ± 1.38µg/mL for hexane extract as compared to the low IC_{50} of standard ascorbic acid suggesting some seaweed extract usually surpass antioxidants in reducing power (Deepak *et al*., 2017).

Our findings are consistent with these observations, as the methanolic extract of *Turbinaria decurrens* showed the strongest FRAP activity among the tested solvents, indicating the presence of highly active compounds (figure 39), likely phenolics, that contributed significantly to the seaweeds reducing power.

4.4.4. Lipid peroxidation assay (LPO): Lipid peroxidation (LPO) assay was performed using concentrations of 0.2, 0.4, 0.6, 0.8, and 1mg/mL.



a) Acetone extract



b) Aqueous extract

c) Methanol extract

Figure 40: LPO assay of all three extracts of *Turbinaria decurrens* (concentration 0.2-1mg/mL)

Table 12: *In-vitro* LPO assay of acetone, aqueous and methanolic extracts of *Turbinaria decurrens*

Concentration (mg/mL)	Acetone (% inhibition)	Aqueous (% inhibition)	Methanol (% inhibition)
0.2	6.38±0.58	5.31±0.44	7.82±0.28*
0.4	9.13±0.43	7.72±0.28	12.27±0.44*
0.6	11.76±0.92	12.46±0.6	17.39±0.57*
0.8	15.29±0.29	14.1±0.57	20.96±0.6*
1	20.97±0.44	17.97±0.86	27.39±0.76*
IC_{50}	2.60	3.08	1.81*

Values expressed as Mean ± SD. Statistical analysis was performed using one-way analysis of variance with post-hoc testing. *= p<0.05

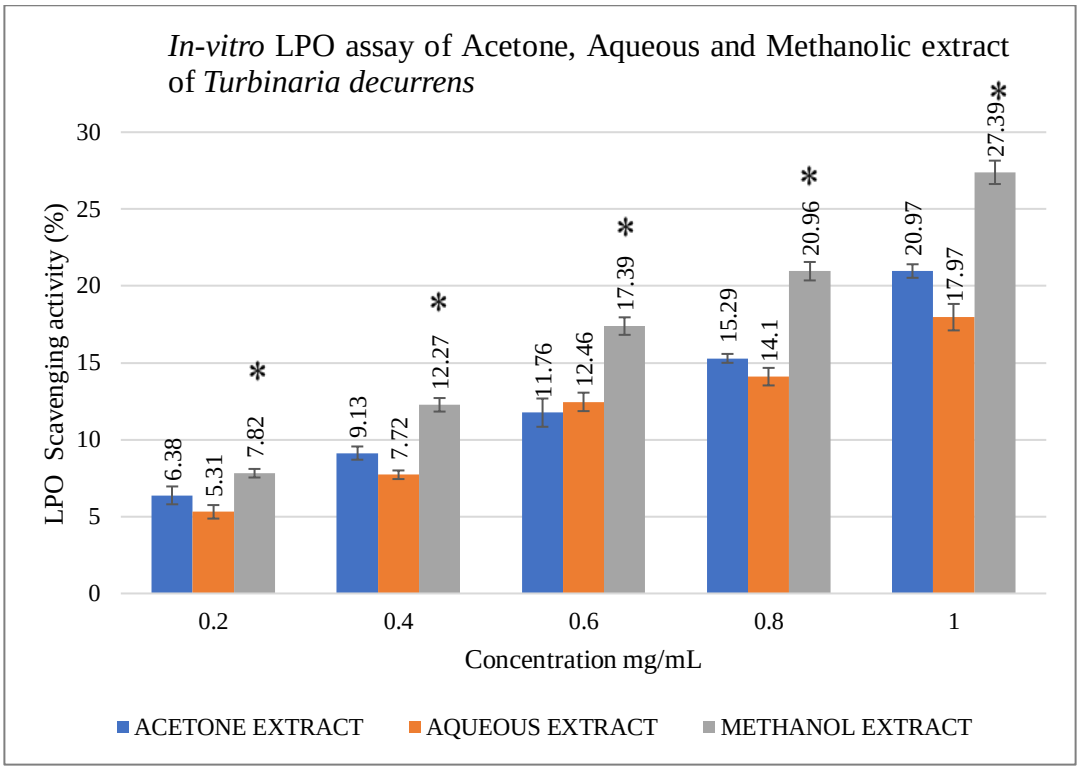


Figure 41: Graphical representation of LPO assay using Acetone, aqueous and methanolic extract of *Turbinaria decurrens*. Statistical analysis was performed using one-way analysis of variance with post-hoc testing. *= p<0.05

Table 13: LPO assay One way ANOVA

ANOVA						
Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	636.8469	4	159.2117	5.720607	0.011652	3.47805
Within Groups	278.3127	10	27.83127			
Total	915.1596	14				

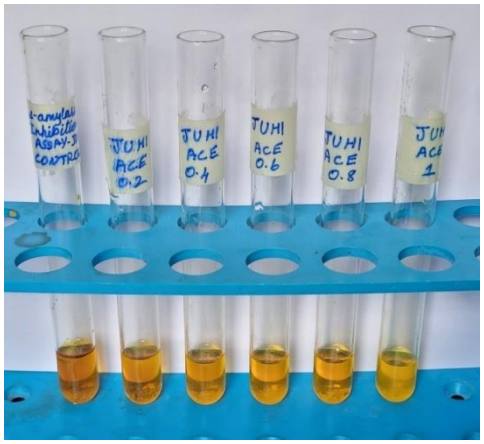
Among the extracts tested, the methanolic extract of *Turbinaria decurrens* exhibited the highest inhibitory concentration with a percentage inhibition of 19.25±11.50% and IC_{50} value of 1.81mg/mL. This was closely followed by the acetone extract, which showed a slightly reduced antioxidant effect of 13.04±6.21% and an IC_{50} 2.60mg/mL. The aqueous extract demonstrated the lowest inhibition with a value of 11.71±5.43% and an IC_{50} 3.08 mg/mL. (Table 12). The results of the LPO assay showed a statistically significant difference (p value < 0.05) among the different solvent extracts.

The present findings closely agree with those given by (Rajauria *et al*., 2013) who also showed that antioxidant activity of methanolic extracts of brown seaweeds increases in a directly proportional manner to the extract concentration. The seaweed *Himanthalia elongata* confirmed that the methanol is a good solvent for extracting peroxidation inhibiting compounds. Similarly, our results revealed that *Turbinaria decurrens* methanolic extract showed higher inhibition compared to acetone and aqueous extract, thus establishing the role of methanol in the isolation of active phenolic compounds responsible for oxidative defense. Furthermore, the methanolic extract higher phenolic and tannin content seems to be correlated with the increased antioxidant activity seen in ABTS, DPPH, FRAP and LPO assays. From a biochemical standpoint phenolic compounds are known to neutralize free radicals and prevent oxidative chain reaction. The DPPH & ABTS radical scavenging activities primarily operates through these mechanisms. The FRAP assay further supports this interpretation. FRAP reflects electron donating capacity indicating the methanolic extract contains compounds capable of reducing Fe^{3+} to Fe^{2+}. The parallel

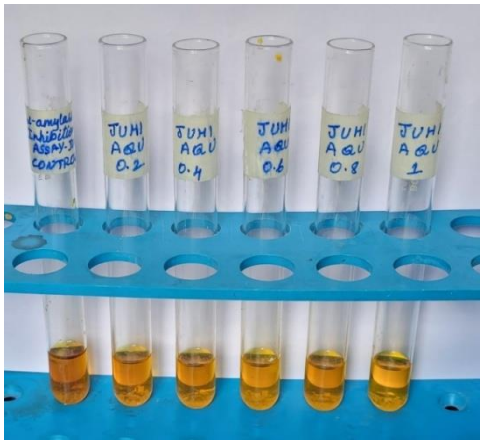
increase in phytochemical concentration and antioxidant assays strengthens the functional association between phytochemical composition and oxidative defense capacity.

4.5 *IN-VITRO* ANTI-DIABETIC ASSAY: The activity of inhibition against α-glucosidase and α-amylase enzymes were investigated using acetone, aqueous and methanolic extract.

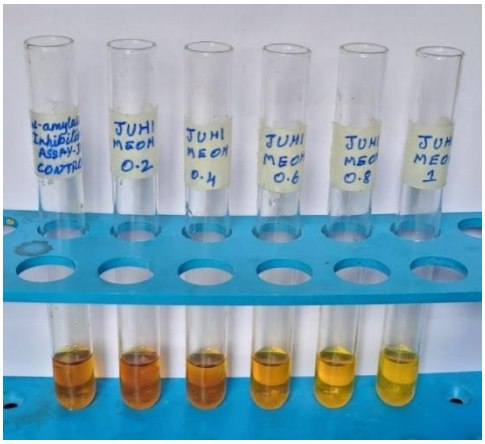
4.5.1 α-amylase inhibitory activity: All extracts demonstrated significant inhibition of α-amylase activity which varied according to the solvent used.



a) Acetone extract



b) Aqueous extract



c) Methanol extract

Figure 42: α-amylase inhibitory assay of all three extracts of *Turbinaria decurrens* (concentration 0.2-1mg/mL)

Table 14: *In-vitro* α-amylase inhibitory assay of acetone, aqueous and methanolic extracts of *Turbinaria decurrens*

Concentration (mg/mL)	Acetone (% inhibition)	Aqueous (% inhibition)	Methanol (% inhibition)
0.2	2.51±0.35	1.61±0.17	3.11±0.27
0.4	6.16±0.27	2.81±0.45	7.14±0.17
0.6	12.5±0.37	5.62±0.27	10.65±0.27
0.8	18.13±0.53	9.51±0.35	27.64±0.35
1	27.34±0.45	12.56±0.35	28.84±0.27
IC_{50}	2.1	4.4	1.8

Values expressed as Mean ± SD

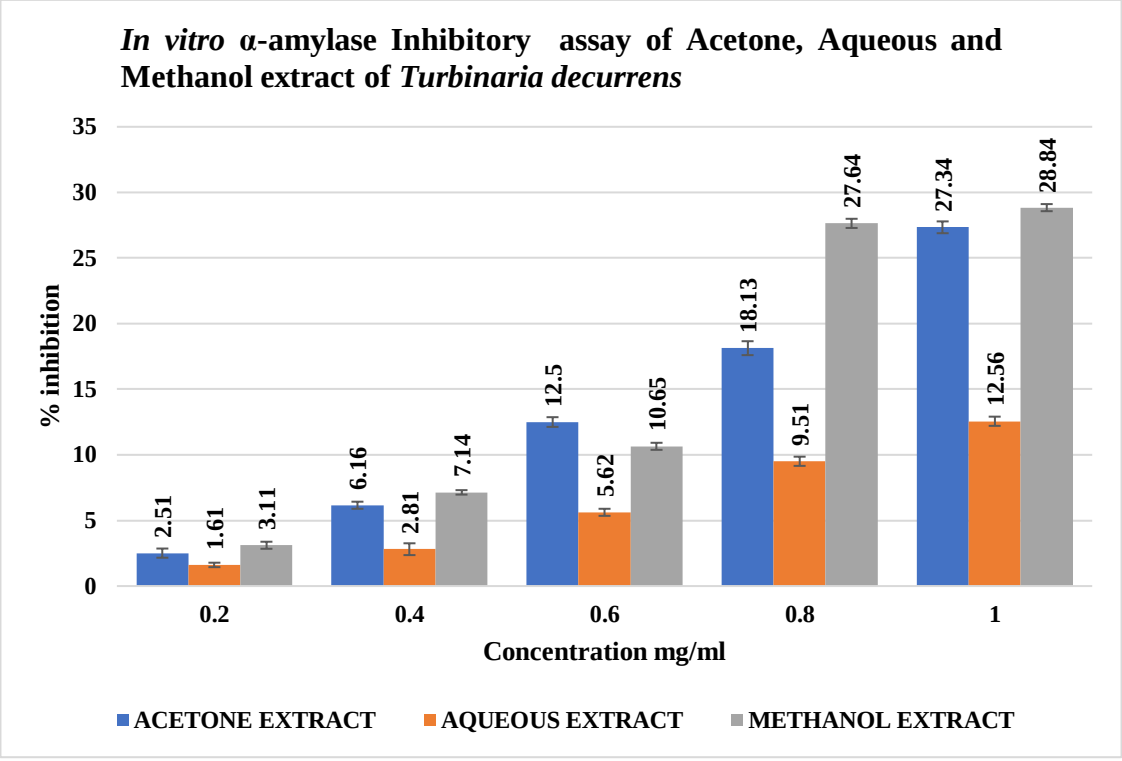


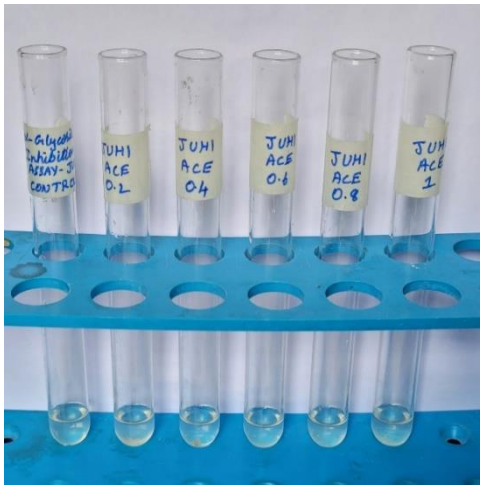
Figure 43: Graphical representation of α-amylase inhibitory assay of Acetone, Aqueous and Methanol extract of *Turbinaria decurrens*

Table 15: α-amylase inhibitory assay one way ANOVA

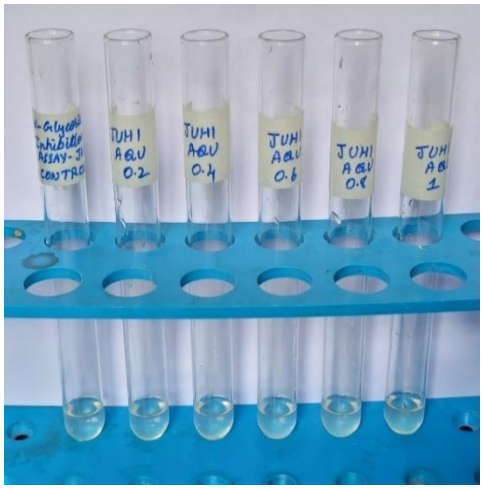
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	960.4978	4	240.1244	6.710314	0.006841	3.47805
Within Groups	357.8438	10	35.78438			
Total	1318.342	14				

Organic solvents exhibited a more pronounced effect compared to aqueous solvents. Notably, methanol extract of *Turbinaria decurrens* displayed the highest inhibition capacity among the three extracts 17.52± 7.45% with an IC_{50} value of 1.8mg/mL followed by the acetone extract and aqueous extract which showed inhibition capacities of 13.43±9.98% & 5.30±4.45% with an IC_{50} value of 2.1 and 4.4 mg/mL respectively (Table 14). This is supported by earlier studies on the edible brown seaweed *Turbinaria ornata,* which demonstrated significant in vitro inhibitory activity against the enzymes α-amylase, α-glucosidase and DPP-IV. Of the various solvent fractions tested, the methanol extract had the greatest α-amylase inhibition even surpassing that of the standard acarbose (Unnikrishnan *et al*., 2014).

4.5.2 α-glucosidase inhibitory activity: The methanolic extract of *Turbinaria decurrens* demonstrated an inhibition rate of 20.94±11.90% and IC_{50} value 1.40mg/mL. In contrast, the acetone extract exhibited 11.61±6.85% inhibition with an IC_{50} 2.63mg/mL while the aqueous extract revealed an inhibitory activity 9.02±5.78% with an IC_{50} 3.26mg/mL (Table 16). The inhibitory activity of *Turbinaria decurrens* against carbohydrate digesting enzymes was clearly influenced by the solvent for extraction.



a) Acetone extract



b) Aqueous extract

c) Methanol extract

Figure 44: α-glucosidase inhibitory assay of all three extracts of *Turbinaria decurrens* (concentration 0.2-1mg/mL)

Table 16: In- vitro α-glucosidase inhibitory assay of acetone, aqueous and methanolic extracts of *Turbinaria decurrens*

Concentration (mg/mL)	Acetone (% inhibition)	Aqueous (% inhibition)	Methanol (% inhibition)
0.2	4.05±0.6	1.56±0.39	5.88±0.39
0.4	5.88±0.39	5.94±0.78	15.16±0.82
0.6	12.15±0.78	8.62±0.78	21.04±0.6
0.8	13.85±0.82	12.54±0.68	26.79±0.6
1	20.76±0.99	15.81±0.82	36.47±0.39
IC_{50}	2.63	3.26	1.40

Values expressed as Mean ± SD

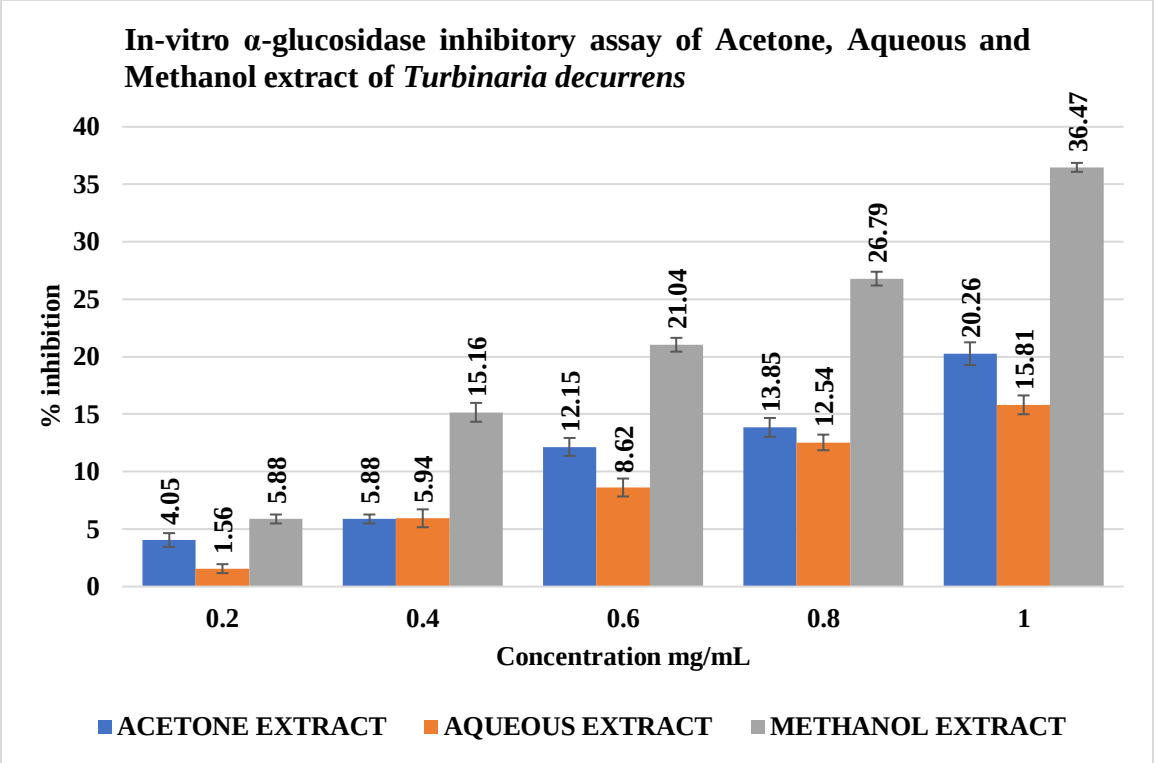


Figure 45: Graphical representation of α-glucosidase inhibitory assay of Acetone, Aqueous and Methanol extract of *Turbinaria decurrens*

Table 17: α-glucosidase inhibitory assay one way ANOVA

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	736.9935	4	184.2484	3.620485	0.045	3.47805
Within Groups	508.9052	10	50.89052			
Total	1245.899	14				

Of the three solvent system investigated, the methanolic extract showed greatest α-glucosidase inhibitory activity followed by acetone and aqueous extracts. These results are in accord with earlier studies involving brown algae, wherein it was shown that polyphenol rich extracts can interfere with carbohydrate metabolism by altering the activity of digestive enzymes (Sapin *et al.*, 2020). The high glycoside content may further contribute to modulation of glucose metabolism while terpenoids may aid in improving metabolic regulation and reducing oxidative stress associated with diabetes. Tannins in particular possess high protein binding affinity, which likely to reduce enzyme catalytic efficiency. Furthermore, *in-vitro* experiment on another brown seaweed *Spatoglossum asperum* proved that methanolic extract showed a strong inhibitory activity against α-amylase and α-glucosidase. At higher concentrations the extract attained over 95% inhibition of α-amylase and almost complete inhibition of α-glucosidase. The IC_{50} value recorded was comparable to pharmaceutical inhibitors and reinforces the functional relevance of its phytochemical richness (Thennarasan, 2015).

4.6 CYTOTOXICITY ASSAY: The evaluation of the extracts of *Turbinaria decurrens* against 3T3 fibroblast cells demonstrated a positive safety profile, as all samples tested showed minimal or no detrimental effects on cell viability. Even at a relatively high concentration of 200µg/mL, the survival rates of the cells remained significantly above 80% for all extracts specifically 84.20% for acetone, 92.10% for aqueous, and 94.12% for methanol. In addition, there were no visible morphological signs of cellular breakdown or cytopathic effects after 48 hours of treatment at greater concentrations (figure 46-49).

The IC_{50} were determined to be 638.81µg/mL (acetone), 1731.91µg/mL (aqueous), and 1309.06µg/mL (methanol), also indicate the low cytotoxic nature of these extracts (Table 18).

Table 18: *In-vitro* cytotoxicity effect of acetone, aqueous, and methanol extracts on 3T3 cell lines.

Sample Concentration (µg/mL)	% Cell Viability		
	Acetone Extract	Aqueous Extract	Methanol Extract
0.0	100.00	100.00	100.00
12.5	96.78	98.79	99.29
25.0	92.99	97.30	98.30
50.0	89.85	95.44	96.67
100.0	85.85	93.88	95.54
200.0	84.20	92.10	94.12
IC_{50}	638.81	1731.91	1309.06

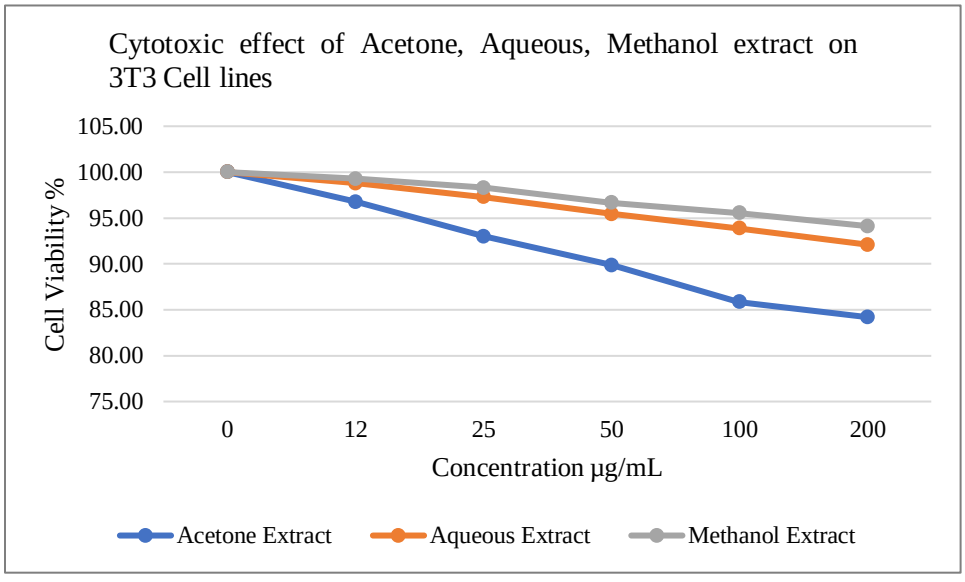
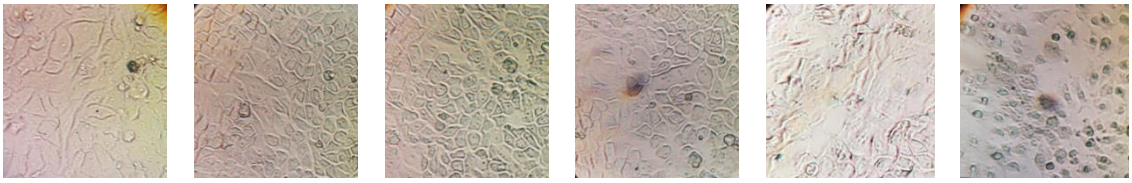
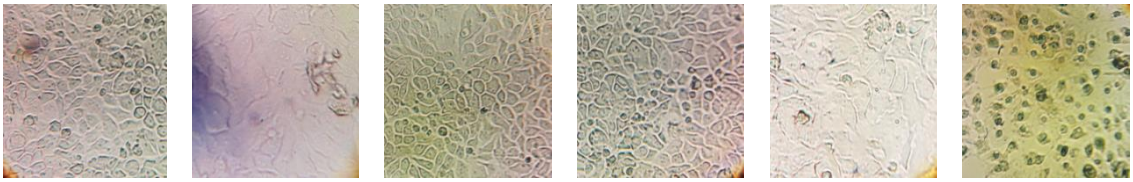


Figure 46: Graphical representation of Cytotoxic effect with Acetone, Aqueous and Methanol extract of *Turbinaria decurrens.*



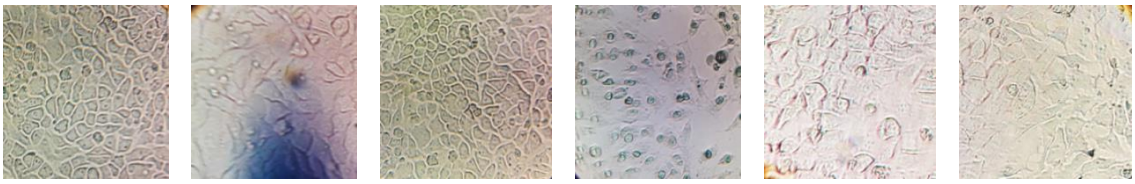
2a 2b 2c 2d 2e 2f

Figure 47: Cytotoxicity effect of *Turbinaria decurrens* Acetone extract sample against 3T3 Cell lines (Images representing different concentration of seaweed sample: (a) control (b) 12.5µg/mL, (c) 25µg/mL, (d) 50µg/mL (e) 100µg/mL (f) 200µg/mL).



3a 3b 3c 3d 3e 3f

Figure 48: Cytotoxicity effects of *Turbinaria decurrens* aqueous extract sample against 3T3 Cell lines (Images representing different concentrations of seaweed sample (a) control (b) 12.5µg/mL, (c) 25µg/mL, (d) 50µg/mL (e) 100µg/mL (f) 200µg/mL).



4a 4b 4c 4d 4e 4f

Figure 49: Cytotoxicity effects of *Turbinaria decurrens* Methanolic extract sample against 3T3 Cell lines (Images representing different concentration of seaweed sample: (a) control (b) 12.5µg/mL, (c) 25µg/mL, (d) 50µg/mL (e) 100µg/mL (f) 200µg/mL).

Our study aligned with earlier research Jeyaprakash *et al*., 2022 which indicated that ethanolic extract of *Turbinaria ornata* demonstrated selective cytotoxic properties against MCF-7 cell lines while leaving normal cells unharmed. Another study showed considerable anticancer activity against HepG2 cell lines (Ponnan *et al*., 2017) further supporting the

presence of bioactive compounds in brown seaweeds exhibiting differential cytotoxic effects. In addition to this, the methanol extract from *Sargassum wightii* tested on Vero cells showed strong cytotoxic effects, while the control group showed no signs of toxicity also supported our study (Gunasekaran *et al*., 2017).

4.7 ANIMAL EXPERIMENTATION: Preliminary investigations have identified the methanolic extract of *Turbinaria decurrens* as the most promising among acetone and aqueous extract. Based on these findings *in-vivo* toxicological analysis was conducted in Wistar rats. The process commenced with acute toxicity study to determine the safe dose for oral administration with no mortality or any significant behavioral changes followed by pharmacodynamic evaluations that indicated a dose dependent response confirming the biological activity.

4.7.1. Acute Toxicity Study:

4.7.1.1. Observation of general signs and behavioral analysis: The safety profile of methanolic extract in Wistar rats was assessed through its oral administration. The animals were divided into four different groups, normal saline (G 1) (10mg/kg body weight) and three varying doses of methanolic extract of *Turbinaria decurrens* extract (50mg, 300mg and 2000mg/kg body weight) was administered in three groups (G 2, G 3 and G 4). The following observations were noted within 24 hours and for 14 consecutive days for general signs of toxicity and behavioral analysis. Animals showed no mortality in any of the groups, no skin and fur abnormalities and also no hyper or reduced activity. The only minor observation was a single instance of lethargy noted on Day 1 in Group 4, but this did not persist in the following days.

Table 19: General appearance and behavioral observations of acute toxicity study for control and subject groups.

Observation	Group 1 (G 1) (Control/Normal Saline 10mg/Kg/BW)			Group 2 (G 2) (*Turbinaria decurrens* methanolic extract 50mg/Kg/BW)			Group 3 (G 3) (*Turbinaria decurrens* methanolic extract 300mg/Kg/BW)			Group 4 (G 4) (*Turbinaria decurrens* methanolic extract 2000mg/Kg/BW)		
	Day 1	Day 7	Day 14	Day 1	Day 7	Day 14	Day 1	Day 7	Day 14	Day 1	Day 7	Day 14
Lethargy or reduced activity	×	×	×	×	×	×	×	×	×	✓	×	×
Loss of appetite	×	×	×	×	×	×	×	×	×	×	×	×
Salivation	×	×	×	×	×	×	×	×	×	×	×	×
Tremors	×	×	×	×	×	×	×	×	×	×	×	×
Diarrhea or vomiting	×	×	×	×	×	×	×	×	×	×	×	×
Disorientation	×	×	×	×	×	×	×	×	×	×	×	×
Skin and fur abnormalities	×	×	×	×	×	×	×	×	×	×	×	×
Hyperactivity	×	×	×	×	×	×	×	×	×	×	×	×
Changes in Urine and feces	×	×	×	×	×	×	×	×	×	×	×	×
Severe weakness	×	×	×	×	×	×	×	×	×	×	×	×
Death	×	×	×	×	×	×	×	×	×	×	×	×

×- Absent, ✓- Present

4.7.1.2. Effect of acute toxicity testing on body weight of animals: The effect of methanolic extract of *Turbinaria decurrens* on body weight was noted weekly. There was a gradual increase in body weight of G 1, G 2, G 3 and G 4 groups. The baseline body weight of all animals was in the range of 150grams – 220grams. The body weight gain from day 1 to day 14 was 6%, 10% and 6 % in G 2, G 3 and G 4 respectively.

Table 20: Effect of *Turbinaria decurrens* methanolic extract on body weight of the animals in different study groups (G 1, G 2, G 3 & G 4) in Acute Toxicity Study

Group	Treatment	Body Weight (grams)		
		Day 1	Day 7	Day 14
1	Control (Normal Saline) (NS)	152.66±3.78	163.33±7.23	184.33±6.80
2	*Turbinaria decurrens* Methanolic extract (50mg/kg/body weight)	159.66±3.51	161.33±3.51	164±5.50
3	*Turbinaria decurrens* Methanolic extract (300mg/kg/body weight)	183±7	187.66±10.26	193±8.54
4	*Turbinaria decurrens* Methanolic extract (2000mg/kg/body weight)	158.66±4.04	161.66±5.03	164.33±5.50

(Values expressed as Mean ± SD; n=3)

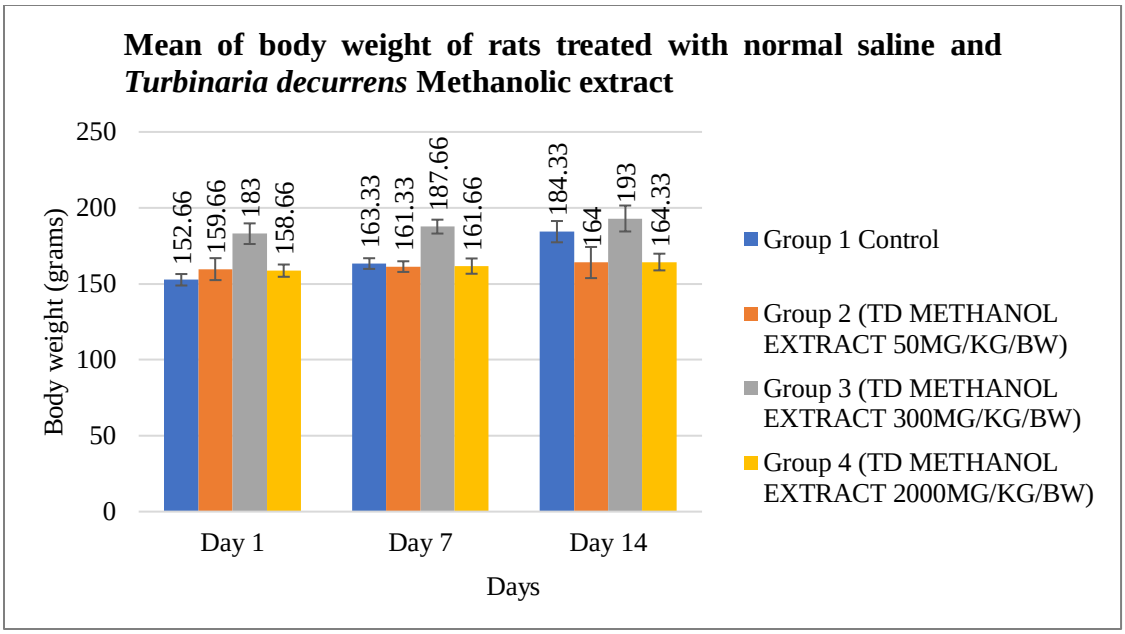


Figure 50: Comparison of mean Body weight of the Group 1 (Control) and Wistar rats treated with different doses of methanol extract of *Turbinaria decurrens* (Group 2, Group 3 & Group 4) during a 14-day Acute Toxicity Study. Values are expressed as Mean ± SD (n=3).

4.7.1.3. Effect of acute toxicity testing on absolute and relative organ weights of animals: The absolute and relative organ weights (ROW) of liver and kidneys for G 1, 2, 3 and 4 were assessed at the conclusion of the study. The gross anatomy of liver and kidney was found to be normal in G 2, G 3 and G 4 when compared with G 1 and there was no significant difference found in the weight of organs in control and treatment groups.

Table 21: Absolute and Relative organ weight of rats in acute oral toxicity study

Parameters	Organ	G 1	G 2	G 3	G 4
Absolute organ weight (grams)	Liver	6.17±0.82	5.74±0.14	6.38 ±0.32	5.89 ±0.09
	Kidney	0.55 ±0.04	0.54±0.04	0.58 ±0.03	0.54 ±0.03
Relative organ weight (grams)	Body weight	184.33±6.80	164±4.58	193±8.54	164.33±5.50
	Liver	3.34 ±0.41	3.51±0.16	3.30 ±0.17	4.13 ±0.27
	Kidney	0.30 ±0.01	0.33±0.02	0.29 ±0.02	0.34 ±0.01

Values expressed as Mean ± SD

4.7.1.4. Effect of acute toxicity testing on Hematological parameters in animals: Routine Hematological assessments namely total white blood cell count, differential leukocyte count (neutrophils and lymphocytes), Hemoglobin, Platelet Count, RBC Count, PCV were conducted on rats administered with *Turbinaria decurrens* extract at doses of 50, 300 & 2000mg/kg body weight. There was no significant difference occurred between the subject and the control group.

Table 22: Effect of acute toxicity testing on Hematological parameters in G 2, G 3, G 4 compared with G 1.

Parameters	G 1	G 2	G 3	G 4
Total WBC Count (10^9/L)	11±1.52	7±0.55	10±2.57	8±2.76
Neutrophils (%)	29.77±10.01	16.71±2.97*	21.65±3.16	23.12±3.32*
Lymphocytes (%)	79.63±4.56	64.96±11.81	79.17±4.27	78.4±6.7
Hemoglobin (Grams %)	13.97±0.15	11.3±0.55	12.2±0.55	11.17±0.72
Platelet Count (10^9/L)	9±0.9	8±0.76	8±1.52	7±0.57
RBC Count (10^12/L)	7.87±0.72	6.90±0.31	7.31±0.25	6.81±0.54
Packed Cell Volume (%)	36.03±1.15	34.8±1.4	36.5±1.8	33.2±2.1

The results were presented as mean ± SD (n=3) Statistical analysis was performed using one-way analysis of variance with post-hoc testing, *=p<0.05

4.7.1.5. Effect of acute toxicity testing on Biochemical parameters in animals: Biochemical analysis of various parameters was expressed in Table 23. The mean value of blood glucose, blood urea, serum creatinine, total cholesterol, serum triglycerides, HDL, LDL, VLDL, SGOT & SGPT did not show any significant differences in G 2, G 3 & G 4 when compared to G 1.

Table 23: Effect of Acute Toxicity Study on Biochemical Parameters in Groups G 2, G 3, G 4 compared with G 1

Parameters	Units	G 1	G 2	G 3	G 4
Blood Glucose	mg/dl	98.33 ± 2.51	99.66± 3.05	101± 4.5	99± 3.0
Blood Urea	mg/dl	32.66 ± 7.57	35.33± 4.72	20.33± 4.51	26.33± 1.90
Serum Creatinine	mg/dl	0.55 ± 0.06	0.58± 0.07	0.54± 0.05	0.76± 0.12
Total Cholesterol	mg/dl	48.83 ± 2.96	50.03± 2.45	59.03± 1.59	61.83± 5.19
Serum Triglycerides (TG)	mg/dl	55.4 ±3.9	56.13± 3.25	55.76± 3.06	58.03± 1.48
High Density Lipoproteins (HDL)	mg/dl	26.46 ± 3.63	26.8± 3.08	30.16± 1.97	28.13± 3.20
Low Density Lipoproteins (LDL)	mg/dl	11.36 ±5.93	12± 5.09	17.7± 0.56	22.61± 3.78
Very Low-Density Lipoproteins (VLDL)	mg/dl	11.04 ±0.8	11.2±0.62	11.15± 0.61	11.14± 0.62
Serum Glutamate Oxaloacetate Transaminase (SGOT/AST)	U/L	81.66±6.65	82.43±7.19	85.5±11.84	95.36±19.72
Serum Glutamate Pyruvate Transaminase (SGPT/ALT)	U/L	72.66±4.04	71.33±3.21	95.13±3.00	109.06±12.22

The results were presented as mean ± SD (n=3) Statistical analysis was performed using one-way analysis of variance with post-hoc testing, *=p<0.05

4.7.1.6. Effect of acute toxicity study on Histopathology of liver and kidneys of animals: In histopathological examination, both, Liver and Kidneys showed normal architecture. Though, lymphocytic infiltration were observed in the kidneys of G 4 that received 2000mg/kg body weight *Turbinaria. decurrens* methanolic extract, they were minimal and comparable with the observations in the control group G 1. While there was absence of congestion, hemorrhages and lymphocytic infiltration in G 2 (50mg/kg) and G 3 (300mg/kg).

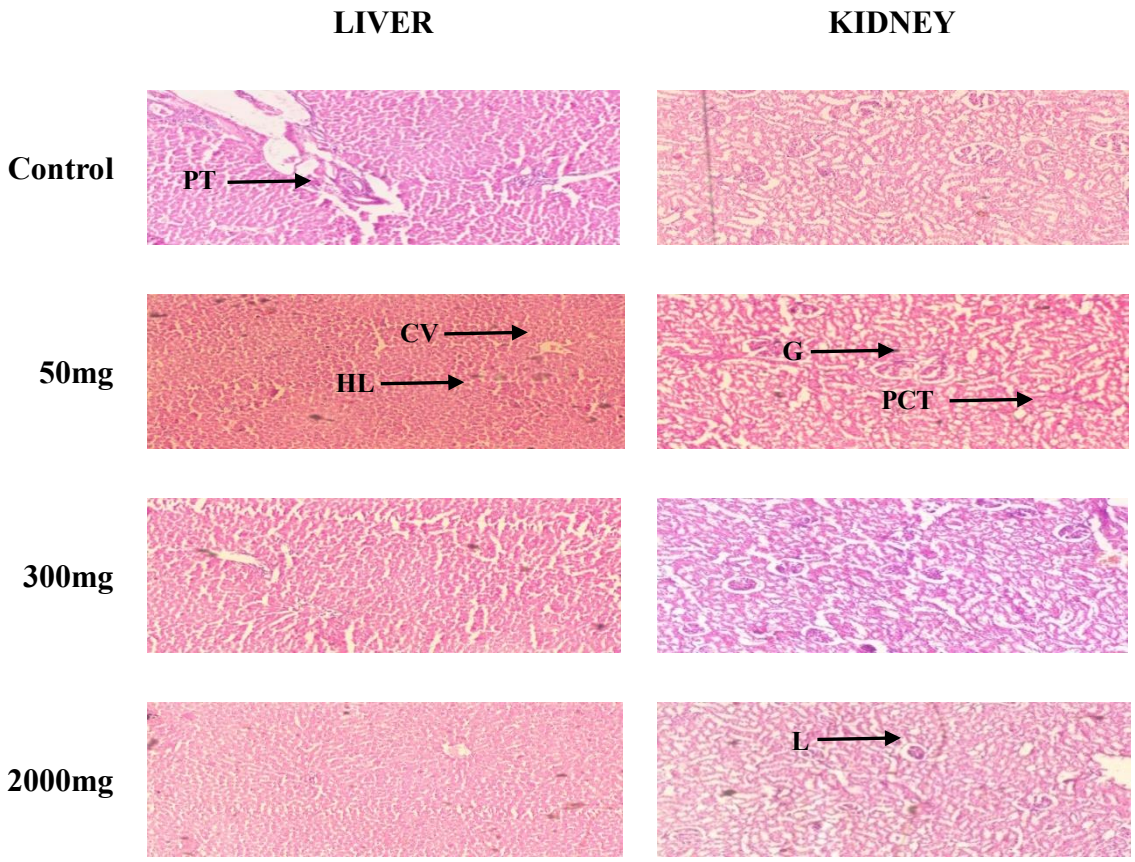


Figure 51: Histopathological observations of Acute Toxicity Study of *Turbinaria decurrens* extract: The histopathological analysis indicated that the normal architecture of the liver and kidneys was maintained in G 2, G 3 & G 4 Wistar rats compared to the G 1. The slides showed PT: Portal triad CV: Central vein, HL: Hepatic lobule, G: Glomeruli, PCT: Proximal convoluted tubule. Lymphocytic infiltration (L) was seen in Kidneys of the G 4 rats while the Liver tissue remained normal.

Acute toxicity investigation evaluated various clinical signs such as appetite, salivation, tremors, diarrhea, disorientation, skin fur condition hyperactivity changes in excreta and overall strength. No significant alterations were seen persisting uniformity throughout Day 1, 7 and 14, mortality was also absent. The body weight of the animals was recorded to check the variation if any as it is utilized as an indicator of potential adverse drug effects (Kharchoufa *et al*., 2020) the weekly recorded data of body weight in this study, demonstrated normal growth physiology, and correlated with proportional food and water consumption at doses of 50, 300, and 2000 mg/kg body weight. Furthermore, a pervasive pain may manifest post-dosing, resulting in a reduced eating rate in the treated rats. Organ weight serves as a primary indicator for assessing targeted organ toxicity (Gwaltney *et al*., 2014). The wet weight of vital organs exhibited no significant differences among groups administered 50, 300, and 2000mg/kg body weight of *Turbinaria decurrens* methanolic extract compared to the normal control, thereby excluding organ-level toxicity of the extract in the selected models. Observations of behavioral patterns in the rats revealed no abnormal indications of motor or sensory processes, even at a high dosage of 2000mg/kg body weight. Consequently, it may be inferred that *Turbinaria decurrens* is mostly harmless when administered orally at a single dose of up to 2000mg/kg body weight. The acute toxicity assessment determined the LD_{50} of *Turbinaria decurrens* to exceed 2000mg/kg body weight, indicating its relative safety as described by the Organization for Economic Cooperation and Development (OECD guidelines). Administration of a chemical compound may bring about significant changes in the structure, function, metabolic transformation, and concentration of biomolecules, enzymes and even metabolic pathways (Agbaje *et al*., 2009). The liver is regarded as the most vital organ due to its role in detoxifying the body. Liver function test primarily includes liver enzymes such as Aspartate Transaminase (AST) and Alanine Transaminase (ALT), which provide insights into the functional status of liver. ALT is a very sensitive biomarker produced in hepatocytes, with increased concentrations noted in circumstances characterized by hepatic inflammation or cellular necrosis. Cellular damage results in the leakage of ALT into the bloodstream, causing an elevation in serum levels. The current investigation found

statistically insignificant changes were observed of ALT and AST levels, thereby negating any potential harmful effects on the liver. These results are further corroborated by findings from the histological examinations of the liver.

Elevated levels of creatinine and urea are strongly associated with renal impairment as they are key indicators of renal damage (Gowda *et al*., 2010). Our investigation demonstrated that the creatinine and urea levels remained within the normal range indicating *Turbinaria decurrens* exerts no adverse effects on renal function. Consequently, it can be inferred that *Turbinaria decurrens* did not disrupt renal physiology or cause any impairment or harm to the kidneys. Despite the observation of minimal lymphocyte infiltration in the kidneys of the group administered 2000 mg/kg of methanolic extract of *Turbinaria decurrens*, no lesions or abnormalities were detected in the gross morphology of the organs, suggesting that *Turbinaria decurrens* exerts no detrimental effects on organ health.

As there are no studies available on the acute toxicity profile of *Turbinaria decurrens,* our results can be compared with those of *Turbinaria conoides* acute toxicity study in Wistar rats. In that study, various extracts (n-hexane, cyclohexane, methanol, and ethanol-water) were administered orally at graded doses. The methanol and ethanol-water extracts did not cause mortality up to a dose of 5g/kg, indicating low toxicity (Kumar *et al*., 2009). The normal blood parameters observed in rats administered with methanolic extract of *Turbinaria decurrens* are a positive indication that these natural substances may potentially be used as a diet supplement for combating blood-related disorders (Azeez *et al*., 2011). Another study of seaweed *Turbinaria decurrens* focused on hepatoprotective effect in ethanol intoxicated rats in which administration of fucoidan from seaweed improve liver functioning by increasing food intake and decreasing hepatic markers. Researchers (Ramu *et al*., 2020) reported that the acute toxicity profile of *Sargassum wightii*, showed no toxic signs or mortality in Wistar rats following a single oral dose of 2000mg/kg body weight, indicating its safety at this level. No adverse effects were found in other biochemical or hematological parameters, and histopathological examinations revealed no signs of toxicity in the vital organs, further supporting the extract's safety profile. The methanolic extract was found to be safe upto 2000mg/kg of body weight of Wistar rats (maximum tolerated

dose by acute toxicity model study as per OECD guidelines). Based on this as well as previous literature three dose levels were selected for the assessment of antidiabetic activity of *Turbinaria decurrens* extract as shown below:

Sample	**Maximum Tolerated Dose (mg/kg)**	**Selected Max Effective Dose (mg/kg)**	**Second Dose (Double the Selected Dose) (mg/kg)**	**Third Dose (Double the Previous Dose) (mg/kg)**
Turbinaria decurrens Methanolic extract	2000	100	200	400

4.7.2. Pharmacodynamic studies: The pharmacodynamic studies included the evaluation of body weight, hematological, biochemical and histopathological evaluation of Wistar rats in different treatment groups (group 1 (G 1) - group 6 (G 6). The rats were divided into six groups (n=8).

4.7.2.1. Effect of treatment on body weight: The baseline body weight of all the rats included in the groups for evaluation of antidiabetic studies were noted and depicted in Table 24.

Table 24: Effect of different doses of Methanol extract of *Turbinaria decurrens* on Body weight in different groups.

Group	Treatment	Body Weight (grams)			
		Day 1	Day 14	Day 28	Body Weight Change
1	Control (Normal Saline) (N.S)	186.3±10.41	185.9±9.2	190.1±10.3	+ 3.75±2.6
2	Streptozotocin (STZ)	162±10.8	120.3±6.0	116±5.3*	-45.6±9.2
3	STZ + Metformin (MF)	153±2.86	166±3.4	168.6±2.96*	+ 15.2±2.3
4	STZ + *Turbinaria decurrens (TD)* Methanol extract (Low dose 100mg/kg)	156±5.45	159±4.9	161.5±4.4*	+ 6.0±2.8
5	STZ + *Turbinaria decurrens (TD)* Methanol extract (Medium (Med.) dose 200mg/kg)	157±6.3	167±7.8	170.8±7.75*	+13±3.9
6	STZ + *Turbinaria decurrens (TD)* Methanol extract (High dose 400mg/kg)	159±4.2	173±4.5	176.7±4.4*	+ 17±2.4

The results are presented as mean ± SD (n=8). Statistical analysis was performed using one-way analysis of variance. *=p<0.05 compared to the diabetic control on day 28.

The diabetic control rats (G 2) showed a remarkable constant loss in body weight by the end of the experiment (-45.6±9.2grams) On the other hand, diabetic rats treated with methanolic extract of *Turbinaria decurrens* (low, medium and high dose) (G 4, 5 and 6) showed a weight gain by + 6.0±2.8grams, +13±3.9grams and + 17±2.4grams respectively. The weight gain was comparable to that of Metformin standard drug treated G 3. Hence it is significant that low, high and medium dose of extracts are capable of protecting the body from significant weight loss in diabetes.

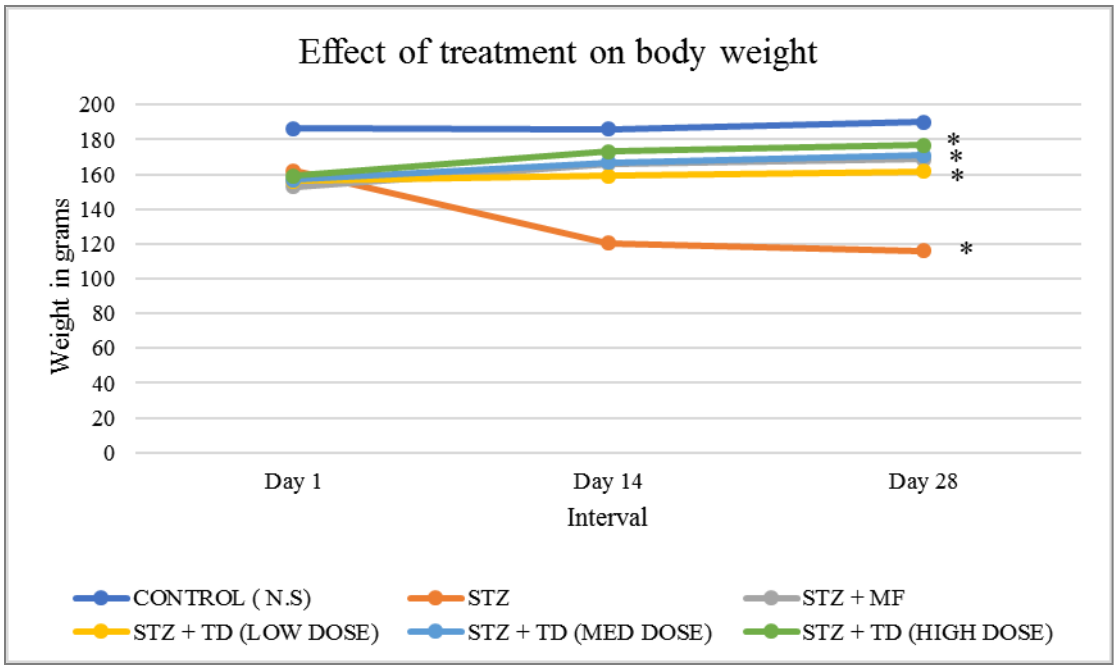


Figure 52: Graphical representation of effects of different doses of methanol extract on body weight in treatment groups compared to controls. Values represented as Mean ± SD (n=8). Statistical analysis was performed using one-way analysis of variance. *= p<0.05 indicates a significant difference compared to the diabetic control.

4.7.2.1.1. Effect of treatment on absolute and relative organ weight: On day 28 of the experimental period, the absolute and relative organ weights were recorded, taking into account of the final body weight of the animals. The organa analyzed included the liver and kidney. Diabetic control animals exhibited significant alteration in absolute (liver 8.0±0.19*, kidney 0.54±0.02) and relative organ weights (liver 6.9±0.33*, kidney 0.46±0.03) compared to the normal control group, reflecting the pathological impact of diabetes (Zafar *et al*., 2010). Treatment with varying doses of methanolic extract of *Turbinaria decurrens* (low, medium and high doses) resulted in dose dependent improvement in both absolute and relative organ weights which suggested that the extract may be exerting protective effect against diabetes induced organ pathology. Our study aligned with Motshakeri *et al*., 2014 that displayed that both ethanolic and aqueous extract of brown seaweed *Sargassum polycystum* can help improve liver and kidney health in a dose dependent manner while also enhancing pancreatic structure in rats suffering from

type 2 diabetes. Statistical analysis using one-way ANOVA revealed that the difference was significant (*p < 0.05).

Table 25: Effect of different doses of Methanolic extract of *Turbinaria decurrens (TD)* on Absolute and Relative organ weights.

Parameters	Organ	G 1 (CONTROL)	G 2 (STZ)	G 3 STZ + MF	G 4 STZ + *TD* LOW DOSE	G 5 STZ + *TD* MED DOSE	G 6 STZ + *TD* HIGH DOSE
Absolute organ weight (grams)	Liver (grams)	10.3±0.12	8.0±0.19*	9.4±0.4*	9.3±0.33*	9.6±0.16*	10.3±0.41*
	RT. Kidney (grams)	0.63±0.12	0.54±0.02	0.5±0.04	0.6±0.09	0.6±0.03	0.61±0.06
Relative organ weight (grams)	Body Weight (grams)	190.1±10.3	116±5.3	168±2.96	161.5±4.4	170.8±7.75	176.7±4.4
	Liver (grams)	5.43±0.26	6.9±0.33*	5.6±0.21	5.76±0.12	5.67±0.2*	5.86±0.1*
	RT. Kidney (grams)	0.33±0.05	0.46±0.03	0.29±0.02	0.41±0.05	0.35±0.01	0.34±0.05

The results are presented as Mean ± SD. Statistical analysis was performed using one-way analysis of variance. *=p<0.05 compared to the diabetic control

4.7.2.2 Effect of different doses of methanolic extract of *Turbinaria decurrens* on biochemical parameters.

4.7.2.2.1 Effect of different doses of methanolic extract of *Turbinaria decurrens* on fasting glucose levels in diabetic rats: The effect of different doses of methanolic extract of *Turbinaria decurrens* on fasting blood glucose levels in diabetic rats were assessed. Diabetic control rats exhibited a significant elevation in fasting glucose levels (381.1±23.7*) compared to the normal control group confirming successful induction of diabetes. Treatment with the methanolic extract of *Turbinaria decurrens* at all tested doses (low, medium and high) resulted in a significant reduction (151.2±17.7*, 123.5±13.2*, 93.1±15.5* respectively) (p < 0.05) in fasting glucose levels compared to the diabetic control group. The group treated with the standard antidiabetic drug also showed a

significant decrease in glucose levels, (102.5±16.7) comparable to that observed with the highest dose of the extract. Despite requiring a higher dose compared to metformin, the extract showed a comparable effect at its highest concentration. The need for higher dose is indicative of the crude phytochemical nature of the extract as opposed to purified pharmacological compound metformin. Thus, while metformin is more potent on a dose for dose basis, the extract had a statistically significant effect size comparable to standard antidiabetic drug. As per the present findings *Turbinaria decurrens* showed a clear dose dependent reduction in fasting glucose levels in diabetic rats which aligned with the antidiabetic effects observed in brown seaweed *Sargassum wightii* (Renitta *et al*., 2020). Similarly brown algae *Padina tetrastromatica* and *Turbinaria conoides* aqueous extract was administered in a dose dependent manner in streptozotocin diabetic Wistar rats led to a notable decrease in fasting glucose levels ($p < 0.01$). The effects were noticeable after the first week and continued to be evident throughout the second week (Kumar *et al*., 2017). These findings suggested that brown algae appear to have the ability to regulate glucose levels.

Table 26: Effect of different doses of Methanolic extract of *Turbinaria decurrens* on fasting glucose levels in Diabetic rats.

Parameter	G 1 (NS)	G 2 (STZ)	G 3 (STZ+MF)	G 4 (STZ + *TD* LOW DOSE)	G 5 (STZ + *TD* MED DOSE)	G 6 (STZ + *TD* HIGH DOSE)
Glucose (mg/dl)	88.1±5.7	381.1±23.7*	102.5±16.7	151.2±17.7*	123.5±13.2*	93.1±15.5*

Values expressed as mean ± SD. Statistical analysis was performed using one-way analysis of variance. *=p<0.05 compared to the diabetic control

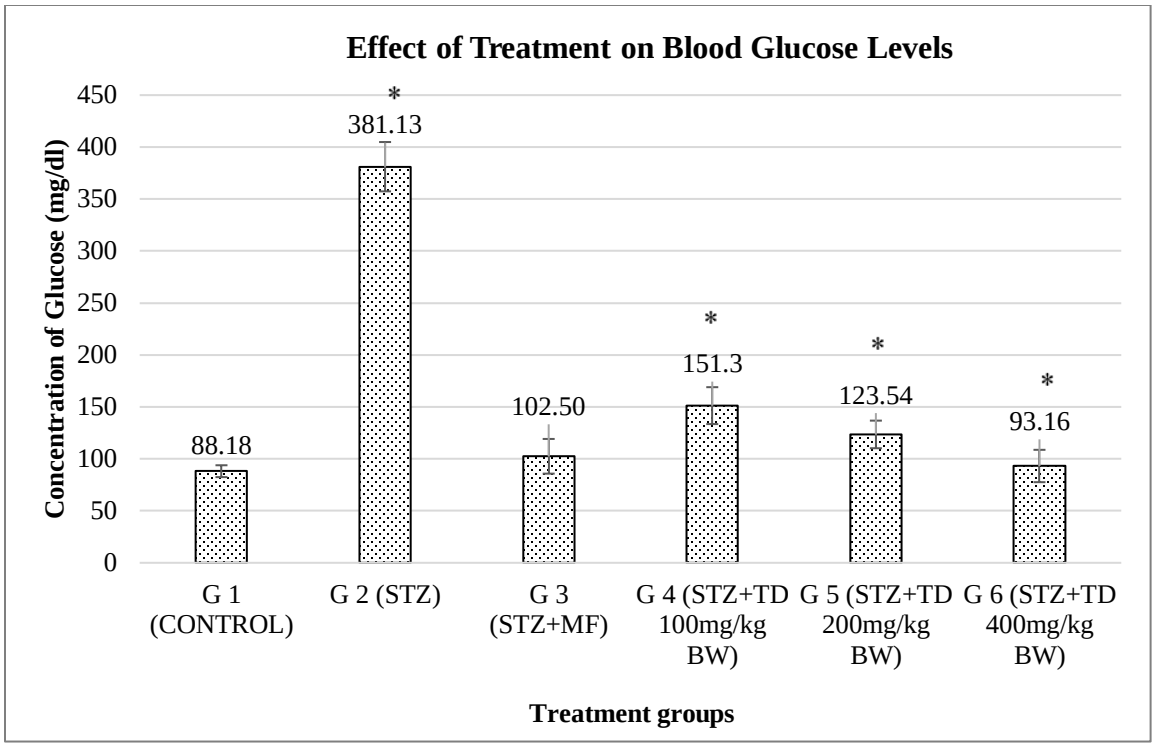


Figure 53: Graphical representation of effect of different doses of methanolic extract of *Turbinaria decurrens (TD)* on blood glucose levels in Wistar rats, Values represented as Mean ± SD (n=8). Statistical analysis was performed using one-way analysis of variance. *=p<0.05 compared to the diabetic control

4.7.2.2.2 Effect of different doses of methanolic extract of *Turbinaria decurrens* on renal function test (urea and creatinine): The effect of various doses of methanolic extract of *Turbinaria decurrens* on kidney function test in diabetic rats, control, and rats treated with different doses of *Turbinaria decurrens* methanolic extract are summarized in Table 27. Diabetic control (G 2) displayed significant elevation in serum creatinine (1.08±0.21mg/dL) and blood urea nitrogen levels (36.7±3.1mg/dL), both of which are markers of renal impairment. Diabetic group receiving metformin (G 3) demonstrated significant reduction in creatinine (0.79±0.09mg/dL) and urea levels (26.6±5.4mg/dL). In contrast, treatment with different doses of *Turbinaria decurrens* methanolic extract resulted in varying degree of improvement in kidney function. Rats receiving the low dose (100mg/kg) (G 4) showed a decrease in creatinine levels (0.83±0.23mg/dL), and urea levels (30.9±6.72mg/dL), while those administered the medium dose (200mg/kg) (G 5) demonstrated a more pronounced reduction in creatinine (0.70±0.12mg/dL) and urea levels

(27.9±6.31mg/dL) which showed slight improvement. The high dose (400mg/kg) (G 6) resulted in creatinine levels (0.84±0.19mg/dL) and urea levels (36.2±11.2mg/dL). Diabetes mellitus is frequently associated with nephropathy, as shown by elevated serum creatinine and blood urea nitrogen levels, which indicate that glomerular filtration and kidney function are compromised. The present study indicated that diabetic control group (G 2) showed notably higher creatinine levels and urea levels reflecting renal impairment linked to hyperglycemia and oxidative stress. These finding aligned with previous studies suggesting that chronic hyperglycemia can lead to glomerular damage and a decrease in the kidney's ability to clear waste (Vallon *et al*., 2011). Treatment with metformin (G 3) showed a significant improvement, resulting in lower levels of both creatinine and urea. The positive effect was likely due to its protective benefit against diabetic nephropathy. The administration of the methanolic extract of *Turbinaria decurrens* to diabetic rats at different concentrations showed dose dependent effects on kidney markers. The low, medium and high (G 4, 5, 6) showed an improvement suggesting a protective effect of methanolic extract seaweed. Our research aligned with Motshakeri *et al*., 2014 that described oral administration of *Sargassum polycystum* extract resulted in a significant reduction in serum urea and serum creatinine levels. Similar results were reported in another study where administration of brown seaweed's aqueous extract reduced the elevated levels of urea and creatinine (P., 2017).

Table 27: Effect of different doses of Methanolic extract of *Turbinaria decurrens* on urea and creatinine levels in Diabetic and control rats.

PARAMETERS	G 1 (N.S)	G 2 (STZ)	G 3 (STZ + MF)	G 4 (STZ + *TD* LOW DOSE)	G 5 (STZ + *TD* MED DOSE)	G 6 (STZ + *TD* HIGH DOSE)
Blood Urea (mg/dl)	19.7±4.4	36.7±3.1*	26.6±5.4	30.9±6.72	27.9±6.31	36.2±11.2
Serum Creatinine (mg/dl)	0.79±0.08	1.08±0.21	0.79±0.09	0.83±0.23	0.70±0.12	0.84±0.19

The results were presented as mean ± SD (n=8). Statistical analysis was performed using one-way analysis of variance. *=p<0.05 compared to the diabetic control

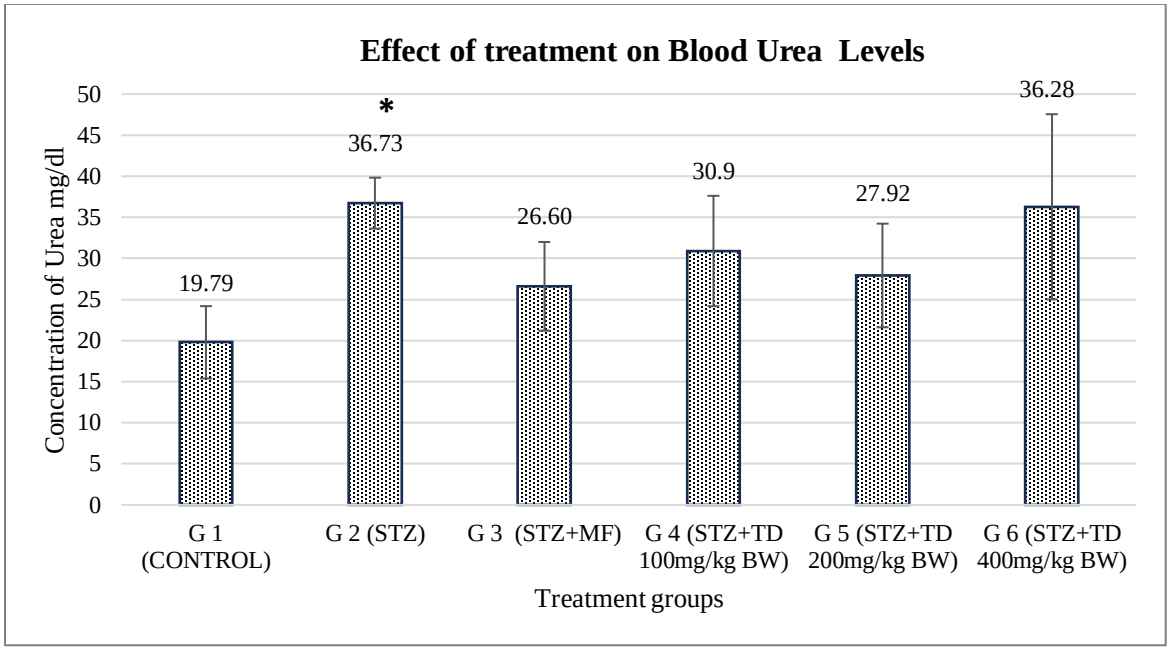


Figure 54: Graphical representation of effect of different doses of methanolic extract of *Turbinaria decurrens* on urea levels in Wistar rats, Values represented as Mean ± SD (n=8). Statistical analysis was performed using one-way analysis of variance. *=p<0.05 compared to the diabetic control.

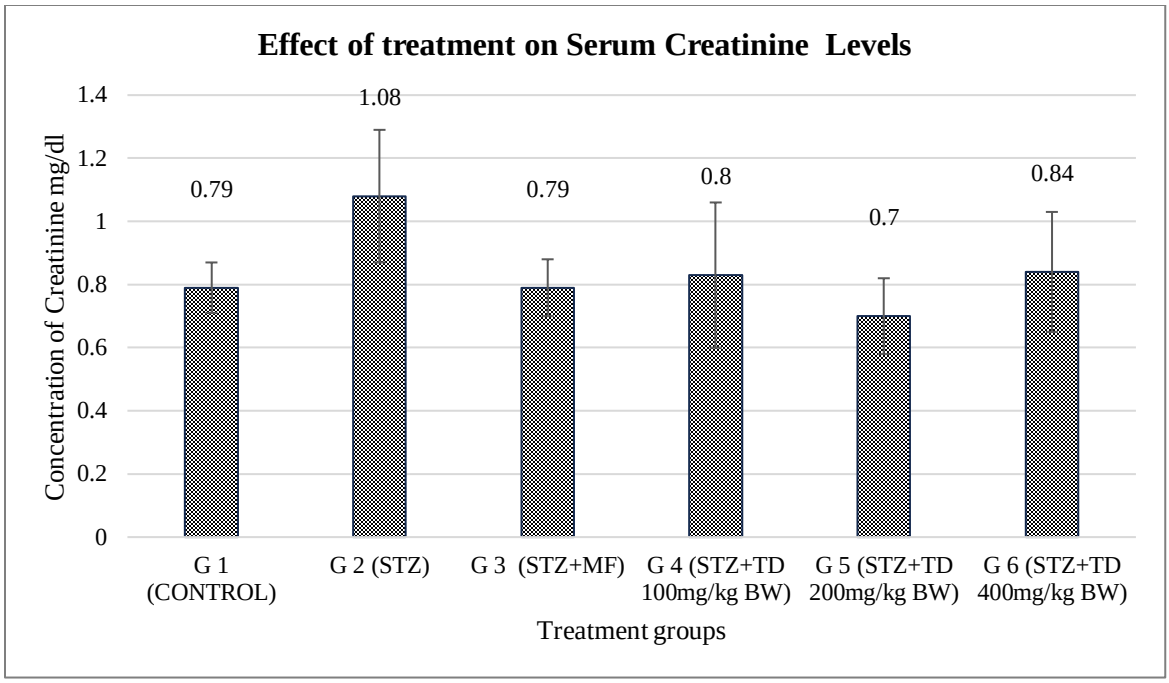


Figure 55: Graphical representation of effect of different doses of methanol extract of *Turbinaria decurrens* on creatinine levels treatment groups compared to controls. Values represented as Mean ± SD (n=8)

4.7.2.2.3 Effect of different doses of methanolic extract of *Turbinaria decurrens* on liver function test SGOT (AST) and SGPT (ALT): Serum markers of liver function specifically alanine transaminase (ALT) and aspartate transaminase (AST) were significantly elevated in the diabetic control (G 2), indicating hepatic damage. The diabetic rats exhibited AST levels of diabetic group were 87.5±2.6U/L and ALT levels of 73.5±6.97U/L. The diabetic group receiving metformin (G 3) demonstrated a significant reduction in AST levels (39.9±7.4U/L) and ALT levels (39.73±6.1U/L). Upon treatment with the methanolic extract of *Turbinaria decurrens* a dose dependent reduction in both ALT and AST levels was observed. Rats received the low dose (100mg/kg) (G 4) of the extract showed a significant decrease in AST levels (59.6±4.9U/L), and ALT levels (55.4±3.3U/L), suggesting some degree of hepatoprotection. In the medium dose group (200mg/kg) (G 5) the AST levels were noted to be 86.5±11.2 U/L and ALT levels were 46.4±14.1U/L which showed a pronounced reduction. The high dose group (400mg/kg) (G 6) exhibited AST levels of 75.3±11.4U/L and ALT levels 41.3±5.3U/L with a marked

improvement compared to the diabetic control group. The levels of ALT and AST serve as important indicators for evaluating liver function and are often used in the early diagnosis of liver issues. Previous studies have shown that when insulin is low the increased activity of aminotransferase enzymes leads to higher rates of ketogenesis and gluconeogenesis, which are common in diabetic conditions. In individual with diabetes, fluctuation in serum AST and ALT levels are closely associated with metabolic dysfunction. The increase in these enzymes among diabetics may be linked to greater release from the liver due to oxidative stress, the accumulation of advances glycation end products and overall liver dysfunction (Qian *et al*., 2015). Streptozotocin, when used to induce diabetes, proved to be harmful and led to damage in liver tissues, linked to increased levels of AST and ALT (Zafar *et al*., 2009). The findings of this study were consistent with previous reports regarding elevated AST and ALT levels under diabetic conditions (Qian *et al*., 2015). Following treatment with algal extract, a notable reduction in serum AST and ALT activities was observed, likely indicating an improvement in liver function. The methanolic extract of *Turbinaria decurrens* appeared to mitigate hepatic damage, as evidenced by the decreased activity of these aminotransferase enzymes in the serum of treated diabetic rats (Abo *et al*., 2023).

Table 28: Effect of different doses of Methanolic extract of *Turbinaria decurrens* on liver parameters SGOT (AST) and SGPT (ALT) in Diabetic and control rats.

PARAMETERS	G 1 (N.S)	G 2 (STZ)	G 3 (STZ + MF)	G 4 (STZ + *TD* LOW DOSE)	G 5 (STZ + *TD* MED DOSE)	G 6 (STZ + *TD* HIGH DOSE)
SGOT (AST) (U/L)	65.2±1.9	87.5±2.6	39.9±7.4	59.6±4.9	86.5±11.2	75.3±11.4
SGPT (ALT) (U/L)	33.49±1.64	73.5±6.97*	39.73±6.1*	55.4±3.3*	46.4±14.1*	41.3±5.3*

The results were presented as mean ± SD (n=8.) Statistical analysis was performed using one-way analysis of variance. *=p<0.05 compared to the diabetic control

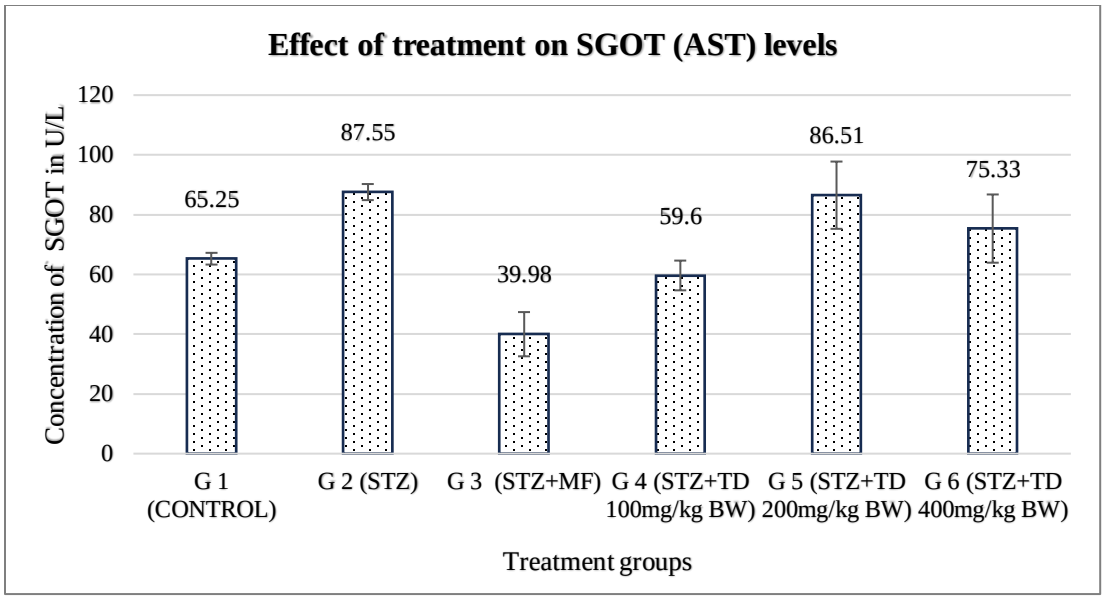


Figure 56: Graphical representation of effect of different doses of methanolic extract of *Turbinaria decurrens* on SGOT levels in Wistar rats, Values represented as Mean ± SD (n=8).

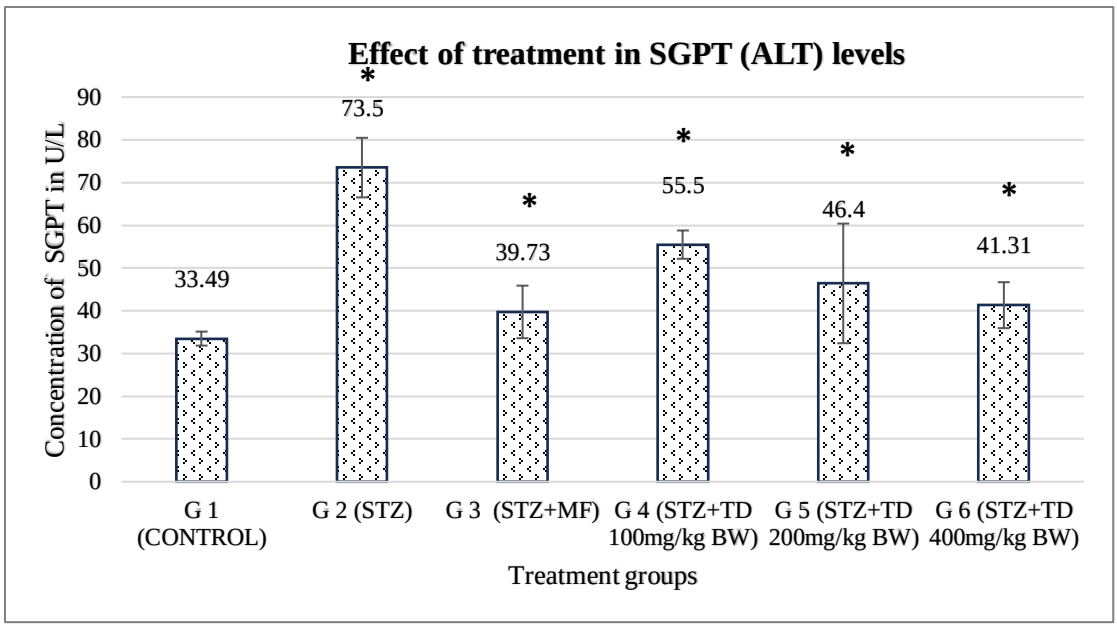


Figure 57: Graphical representation of effect of different doses of methanolic extract of *Turbinaria decurrens* on SGPT levels in Wistar rats, Values represented as Mean ± SD (n=8). Statistical analysis was performed using one-way analysis of variance. *=p<0.05 compared to the diabetic control

4.7.2.2.4 Effect of different doses of methanolic extract of *Turbinaria decurrens* on lipid profile: The lipid profile was assessed in rats across experimental groups, including those that received the test drug, a standard drug, normal treatment, and streptozotocin-induced conditions. The parameters measured in the lipid profile included Total Cholesterol (TC), Triglycerides (TG), High-Density Lipoprotein (HDL), Very Low-Density Lipoprotein (VLDL), Low-Density Lipoprotein (LDL), groups 1 - 6, respectively.

Table 29: Effect of different doses of methanol extract of *Turbinaria decurrens* on Lipid Profile in diabetic rats, with comparison to normal and diabetic control groups.

PARAMETERS	G1 (N.S)	G2 (STZ)	G3 (STZ + MF)	G4 (STZ + *TD* LOW DOSE)	G5 (STZ + *TD* MED DOSE)	G6 (STZ + *TD* HIGH DOSE)
Total Cholesterol (mg/dl)	133.87±5.22	177.81±5.95	132.6±20	128±14.7	124±18.6	115.8±9.7*
Triglycerides (mg/dl)	118.98±13.01	128.39±9.20	120.7±9	113.6±23.2	110.6±7.5	97.1±4.6*
HDL Cholesterol (mg/dl)	56.22±1.84	32.4±3.32	32.6±4.5	39.3±5.60*	42.2±5.5*	46.4±2.2*
LDL Cholesterol (mg/dl)	53.86±4.89	89.7±6.38	75.8±15.5	72.03±16.8	59.4±19.2	49.9±10.9*
VLDL Cholesterol (mg/dl)	23.80±2.60	25.67±1.83	24.1±1.8	22.7±4.65	22.3±1.5	19.4±0.9*

The results were presented as mean± SD (n=8.) Statistical analysis was performed using one-way analysis of variance. *=p<0.05 compared to the diabetic control

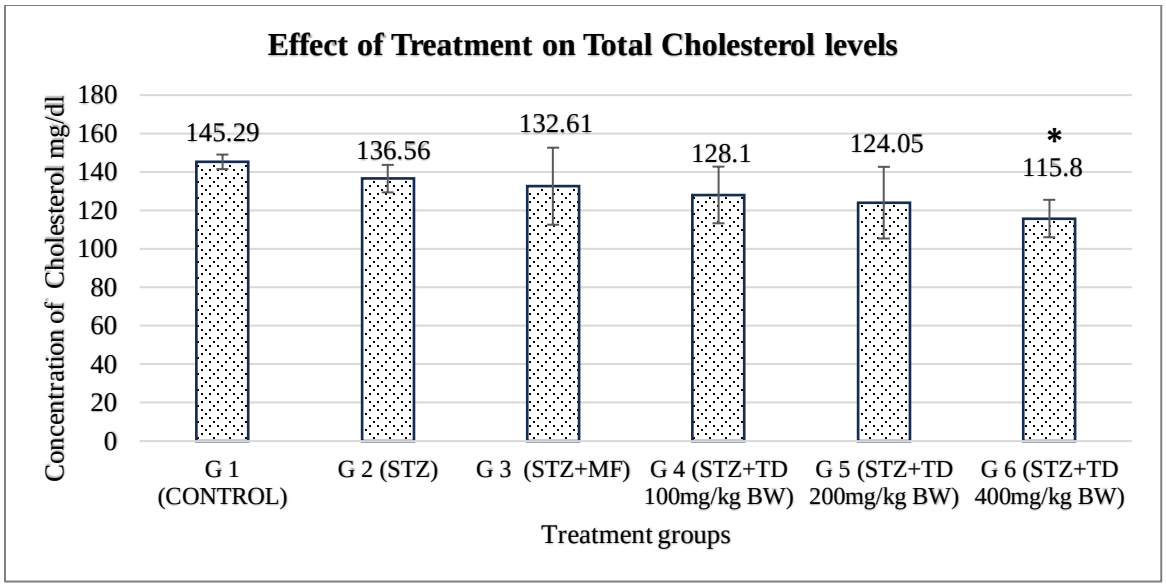


Figure 58: Graphical representation of effect of different doses of methanolic extract of *Turbinaria decurrens* on Total Cholesterol levels in Wistar rats. Values represented as Mean ± SD (n=8). Statistical analysis was performed using one-way analysis of variance. *=p<0.05 compared to the diabetic control

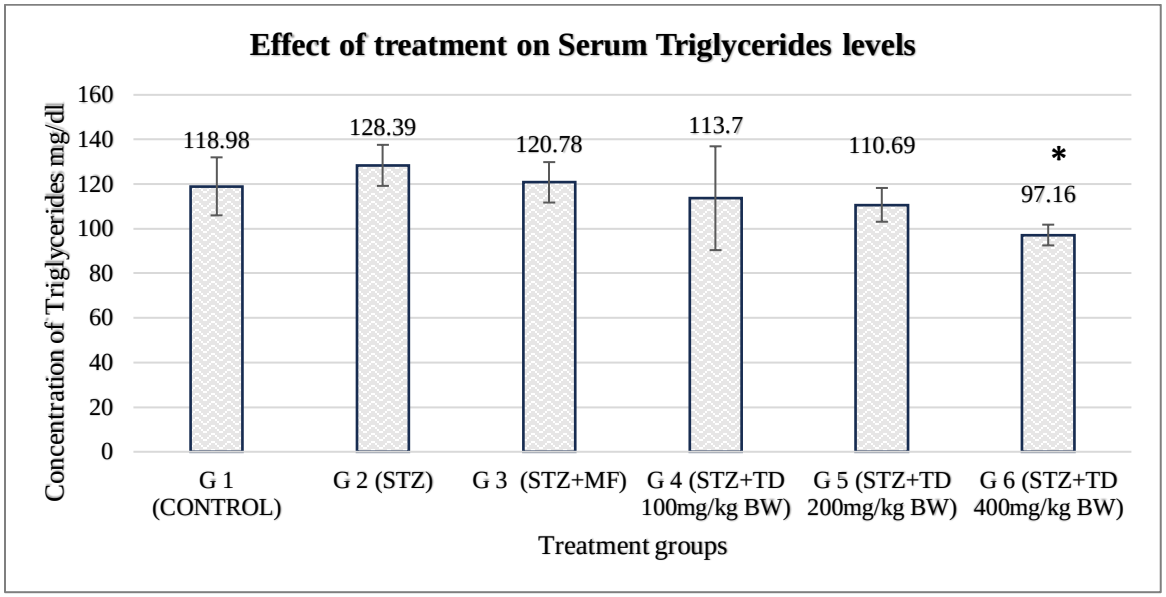


Figure 59: Graphical representation of effect of different doses of methanolic extract of *Turbinaria decurrens* on Serum Triglycerides levels in Wistar rats, Values represented as Mean ± SD (n=8). Statistical analysis was performed using one-way analysis of variance. *=p<0.05 compared to the diabetic control.

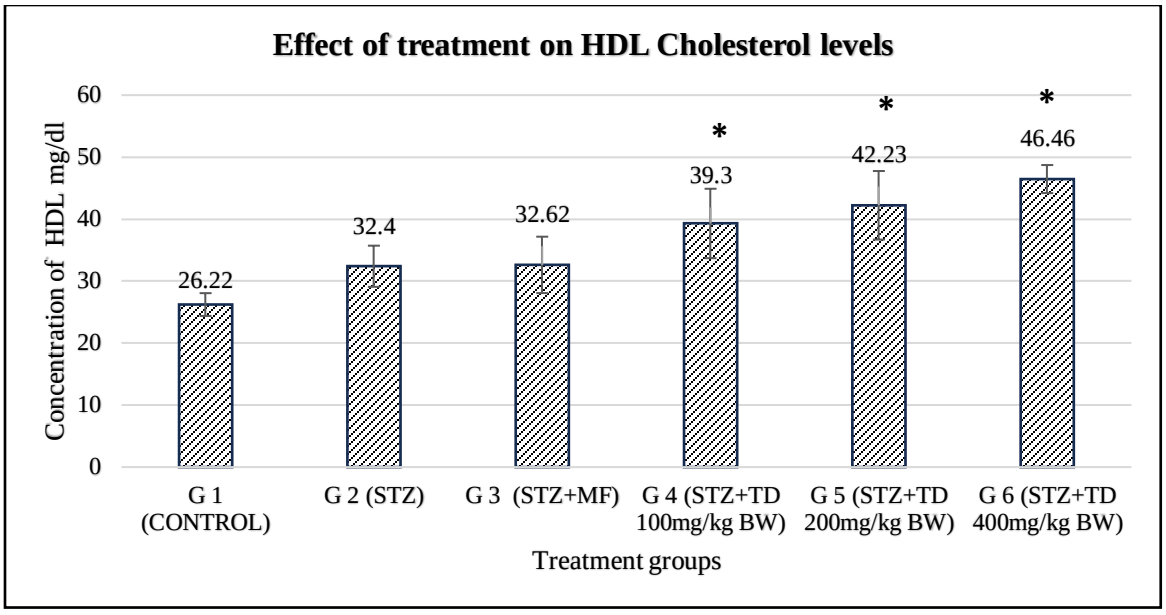


Figure 60: Graphical representation of effect of different doses of methanolic extract of *Turbinaria decurrens* on HDL levels in Wistar rats, Values represented as Mean ± SD (n=8). Statistical analysis was performed using one-way analysis of variance. *=p<0.05 compared to the diabetic control

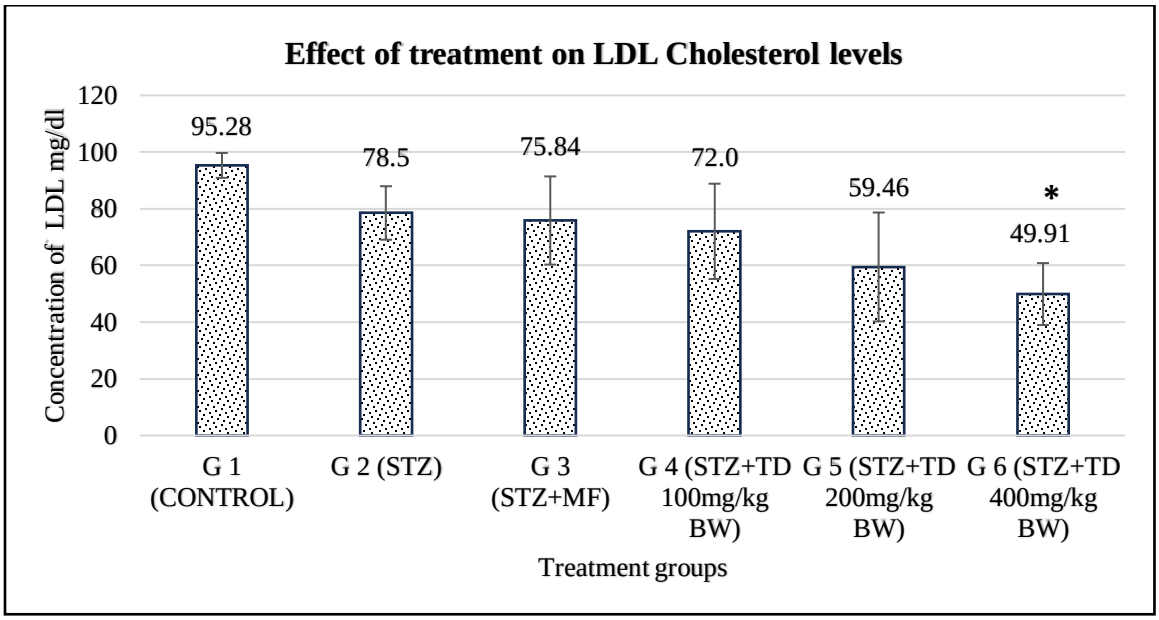


Figure 61: Graphical representation of effect of different doses of methanolic extract of *Turbinaria decurrens* on LDL levels in Wistar rats, Values represented as Mean ± SD (n=8). Statistical analysis was performed using one-way analysis of variance. *=p<0.05 compared to the diabetic control

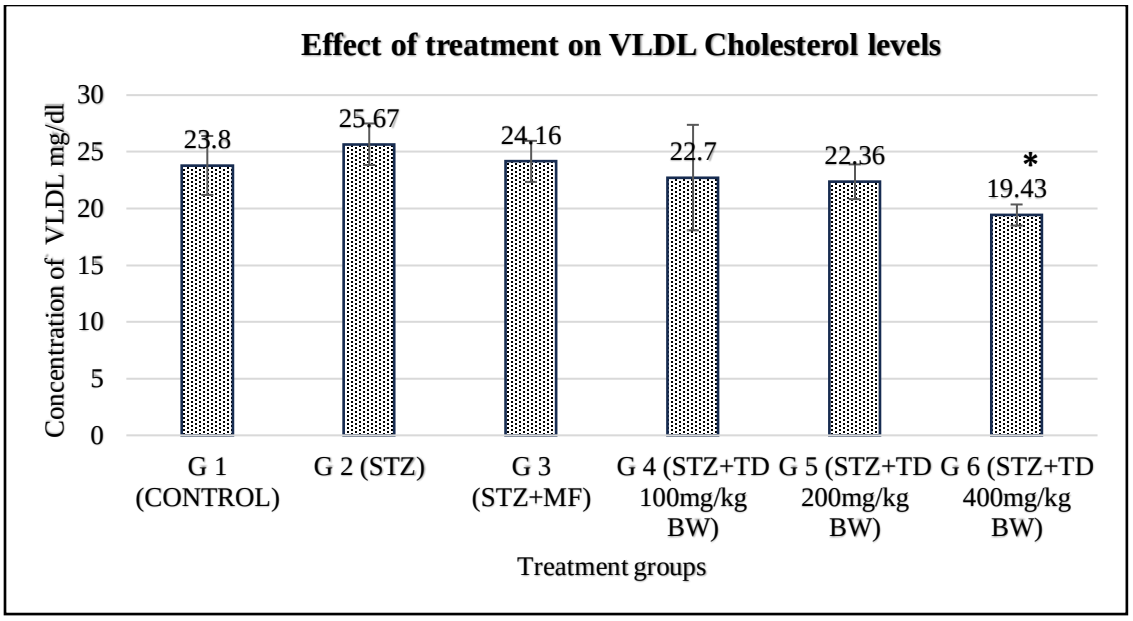


Figure 62: Graphical representation of effect of different doses of methanolic extract of *Turbinaria decurrens* on VLDL levels in Wistar rats, Values represented as Mean ± SD (n=8). Statistical analysis was performed using one-way analysis of variance. *=p<0.05 compared to the diabetic control.

The total cholesterol levels in G 2 were found to be 177.81±5.95mg/dl, triglycerides 128.39±9.20mg/dl, HDL 32.4±3.32mg/dl, LDL 89.7±6.38mg/dl & VLDL 25.67±1.83mg/dl. In G 3 the total cholesterol levels were found to be 132.6±20.0 mg/dl, triglycerides 120.7±9.0mg/dl, HDL 32.6±4.5mg/dl, LDL 75.8±15.5mg/dl & VLDL 24.1±1.8mg/dl. Upon treatment with varying concentration of *Turbinaria decurrens* methanolic extract the G 4 to G 6 showed reduction in the total cholesterol, triglycerides, LDL and VLDL levels as compared to G 2 while HDL levels were elevated in a dose-dependent manner. In conditions of diabetes, inadequate insulin function allows for increased lipolysis and heightened serum lipid levels, which contribute to the risk of cardiovascular disease (Shah *et al*., 2014). In the normal states, insulin triggers the activity of lipoprotein lipase, breaking down triglycerides. In diabetic conditions, however the lack of insulin inhibits the activation of the enzyme, leading to higher levels of triglycerides in the blood (Gao *et al*., 2024). Moreover, the activation of lipoprotein lipase and lecithin acyl cholesterol transferases resulted in a rise in the levels of LDL cholesterol particles (Kwiterovich, 2000). At the same time, the increased amounts of VLDL and triglycerides

were linked to drop in HDL levels. In our study, administration of various doses of the methanolic extract of *Turbinaria decurrens* represented a notable reduction in lipid profile of diabetic rats. Furthermore, a notable reduction in triglycerides, LDL levels were observed while HDL levels in serum of group 4,5 and 6 were increased. These changes can likely be linked to the bioactive compounds present in seaweeds such as alkaloids, which are known for their antioxidant properties. These compounds may play a key role in lowering cholesterol (Fung *et al*., 2013). These findings were in agreement on the methanolic extract of *Sargassum teneriimum* which retained the lipid profile in the diabetic rats by reducing the levels of TG, TC and LDL meanwhile, increasing the level of HDL cholesterol (Lindsey *et al*., 2021).

4.7.2.3 Effect of different doses of methanolic extract of *Turbinaria decurrens* on antioxidant enzymes in liver and kidney: The activities of antioxidant enzymes and lipid peroxidation levels were assessed in tissues of control and experimental groups (Table 30).

Table 30: Effect of different dose of methanolic extract of *Turbinaria decurrens* on enzyme activities and lipid peroxidation in Streptozotocin diabetic rats

Parameters	G 1 (Control)	G 2 (STZ)	G 3 (STZ + MF)	G 4 (STZ + *TD* LOW)	G 5 (STZ + *TD* MED)	G 6 (STZ + *TD* HIGH)
Catalase (Liver) µmol/minute/ mg protein	23.08±0.49	14.18±0.54	21.07±0.39	18.34±0.64	20.25±0.52	20.77±0.42
Catalase (Kidney) µmol/minute/ mg protein	33.67±0.05	21.57±0.04	31.26±0.09	29.04±0.05	29.77±0.02	30.35±0.07
Glutathione Peroxidase (Liver) µmol/minute/ mg protein	14.54±0.06	6.54±0.04	13.84±0.08	9.67±0.06	11.42±0.05	12.97±0.05
Glutathione Peroxidase (Kidney) µmol/minute/ mg protein	5.98±0.01	2.57±0.01	4.41±0.02	3.12±0.04	3.09±0.04	4.01±0.05

Superoxide Dismutase (Liver) µmol/minute/ mg protein	8.54±0.15	4.23±0.02	6.85±0.04	5.95±0.38	6.04±0.07	6.98±0.06
Superoxide Dismutase (Kidney) µmol/minute/ mg protein	13.42±0.02	6.07±0.07	11.68±0.08	10.02±0.06	10.58±0.04	11.76±0.07
LPO (Liver) n/mol/mg protein	0.95±0.01	2.49±0.05	0.89±0.07	0.70±0.02	0.83±0.05	0.87±0.02
LPO (Kidney) n/mol/mg protein	0.85±0.04	1.95±0.07	0.81±0.04	0.69±0.04	0.73±0.05	0.79±0.01

The results are presented as mean ± SD (n=8)

In diabetic control group G 2 a notable drop in the activities of catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase in both the liver and kidney when compared to the normal control group (G 1) was seen. Specifically, CAT activity decreased from 23.08±0.49 to 14.18±0.54 µmol/min/mg protein in the liver and from 33.67±0.05 to 21.57±0.04 µmol/min/mg protein in the kidney. Likewise, GPx and SOD levels were significantly reduced in the diabetic rats, while LPO levels were increased in both tissues' liver 2.49±0.05 to 0.95±0.01n/mol/mg protein in control; kidney 1.95±0.07 to 0.85±0.04n/mol/mg protein. However, treatment with metformin (G 3) and *Turbinaria decurrens* extract (G 4 - G 6) led to improvement in antioxidant enzyme activities and a decrease in LPO levels. The medium dose and high dose proved to be more effective than the low dose. LPO levels decreased across all treatment groups of extract suggesting a reduction in oxidative damage. A well-known factor in diabetic complications is oxidative stress primarily due to production of reactive oxygen species (Sarmento *et al*., 2013). This research presented a decline in antioxidant enzymes namely CAT SOD and GPx in the liver and kidney of the diabetic control group while increasing the LPO. These findings aligned with previous research indicating that hyperglycemia disrupts the antioxidant defenses resulting in oxidative harm to essential organs (Asmat *et al*., 2016). The administration of *Turbinaria decurrens* extract has shown an ability to counteract these changes. The

antioxidant activity restoration showed the protective effect of bioactive compounds present in *Turbinaria decurrens*. These protective effects were consistent with earlier research, highlighted the antioxidant and antidiabetic properties of brown algae.

4.7.2.4 Effect of different doses of methanolic extract of *Turbinaria decurrens* on hematological parameters Hb, RBC, Neutrophils, Lymphocytes and platelets:

The hematological parameters of the experimental groups were summarized in Table 31.

Table 31: Effect of different dose of methanolic extract of *Turbinaria decurrens* on hematological parameters in Streptozotocin diabetic rats.

Treatment Group	G 1 (CONTROL)	G 2 (STZ)	G 3 (STZ+MF)	G 4 (STZ +*TD* LOW DOSE)	G 5 (STZ+*TD* MED DOSE)	G 6 (STZ +*TD* HIGH DOSE)
Hemoglobin (gm%)	14.5± 0.58	15.4±0.25	15.0±0.68	15.2± 0.5	14.5 ± 0.94	14.6 ± 0.6
WBC (10^9/L)	7.7 ± 1.18	11.0±1.6*	9.45±0.97	8.4 ± 0.4*	8.18 ± 0.44*	8.0±0.69*
Lymphocytes (%)	73 ± 3.6	80 ± 2.3	78 ± 1.5	77 ± 1.0	75 ± 3.0	75 ± 0.5
Neutrophils (%)	24 ± 3.0	19.5± 1.5*	21.5 ± 1.3	22.7 ± 1.0	22.5 ± 1.7	22.5±1.9*
RBC (10^{12}/L)	8.6 ± 0.4	8.54 ± 0.4	8.6 ± 0.2	8.5 ± 0.4	8.37 ± 0.3	8.5 ± 0.53
Platelets (10^9/L)	10.3 ± 1.8	10.0 ± 0.66	10.2 ± 1.5	9.8 ± 1.1	9.93 ± 0.8	10.04±1.0

The results are presented as mean ± SD (n=8.) Statistical analysis was performed using one-way analysis of variance. *=p<0.05 compared to the diabetic control

Hemoglobin concentration, RBC count and platelet levels showed no significant differences among the control, STZ and treatment groups, indicating that neither STZ administration nor the treatments produced major alterations in these indices. In contrast, marked changes were observed in the leukocyte profile. The STZ induced diabetic group (G 2) exhibited a significant increase in total WBC count compared with the control group (G 1), reflecting an enhanced inflammatory response. Treatment with metformin (G 3) and *TD* at low, medium and high doses (G 4 - G 6) resulted in a reduction in WBC

count compared to the STZ group, suggesting an attenuation of STZ- induced leukocytosis. Differential leukocyte counts also demonstrated STZ mediated alterations. Lymphocyte percentage increased in the STZ group relative to controls whereas neutrophils percentage decreased. These changes were partially normalized in all treatment groups, with lymphocyte and neutrophils values approaching those of the control animals.

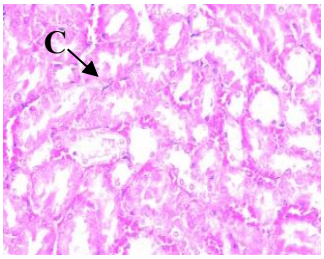
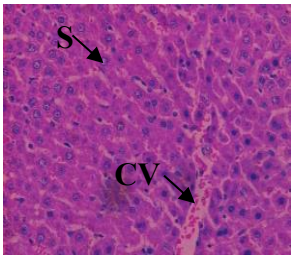
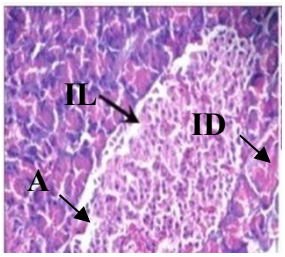
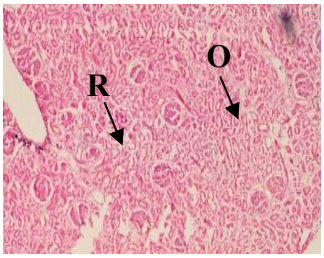
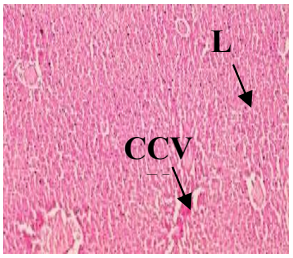
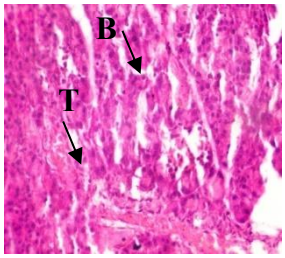
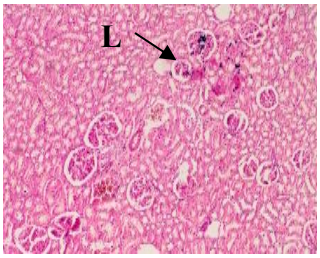
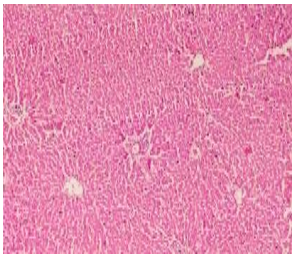
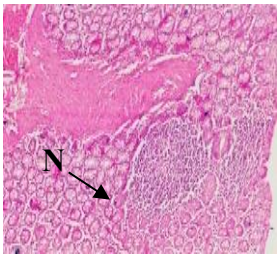
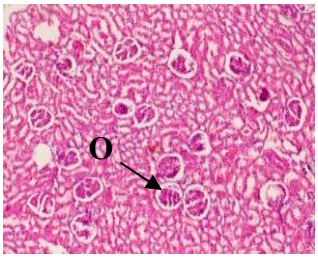
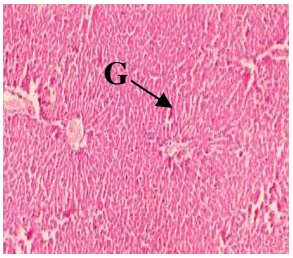
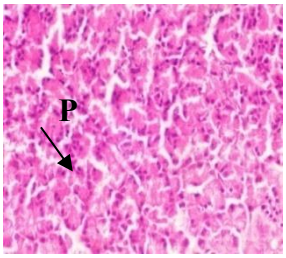
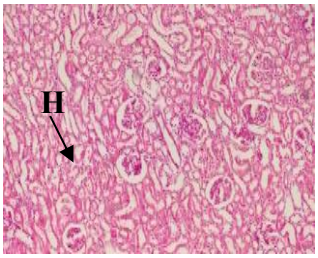
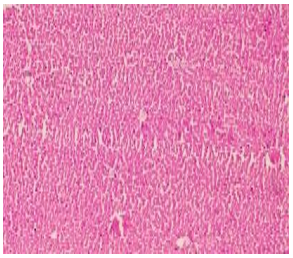
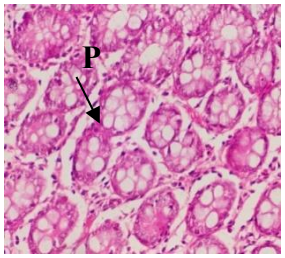
4.7.2.5 Effect of different doses of methanolic extract of *Turbinaria decurrens* on histopathology of liver, kidney and pancreas: Histopathological analysis of the kidney, liver and pancreas provided important insights into the protective and restorative potential of *Turbinaria decurrens* extract against streptozotocin induced diabetic damage.

The histological appearance of the liver tissue sections of rats in the control group (G 1) revealed normal hepatic architecture (figure 63). Experimental group's G 2 liver showed pronounced alterations including, sinusoidal congestion as well as congestion and dilation in the central vein with lymphocytic infiltration. These pathological changes were in line with hepatic metabolic stress associated with diabetes (Gjorgjieva *et al*., 2019). These alterations started to reduce in metformin treated G 3exhibited moderate recovery of liver histoarchitecture. Rats treated with methanolic extracts of *Turbinaria decurrens* (G 4, 5, and 6 in figure 63), demonstrated improved cellular arrangement while as medium and high doses of methanolic extracts of *Turbinaria decurrens* provided better inflammation prevention. Hepatoprotective activity of *Hydroclathrus clathratus* against damage of liver tissue was noticed by (Nagy, 2015). Previous investigations have reported that diabetic rats exhibited severe hepatic alterations such as hepatocellular necrosis which were markedly improved following treatment from ethanolic extracts of *Turbinaria conoides,* methanolic extract of *Sargassum wightii* (Rayapu *et al*., 2017).

Histological examination of sections from pancreas further confirmed the beneficial effects of *Turbinaria decurrens* extracts. The control group G 1 showed normal acinar parenchyma of pancreas along with ducts and islets of Langerhans (figure 63). G 2

pancreatic tissue showed damage to the acini reflecting streptozotocin induced pancreatic cytotoxicity (Khin *et al*., 2023). Tissue was not intact and lymphocytic infiltration was seen. In experimental G 3, the architecture was maintained with acini present. Normal Acinar parenchyma was found to be maintained and regeneration of islets of Langerhans was observed in G 4, 5, and 6 with methanolic extracts of *Turbinaria decurrens.* The group treated with methanolic extracts of *Turbinaria decurrens* exhibited healing characteristics. More protection was reported in medium and high doses of. *Turbinaria decurrens* methanolic extracts. In line with these findings, other brown seaweeds such as *Sargassum tenerimum* have been reported to enhance pancreatic recovery by stimulating regeneration of β cells and reducing necrotic changes associated with diabetic induction (Lindsey *et al*., 2021).

Histological examination of sections from the kidneys of G 1 showed normal glomerular structure (figure 63). In contrast the G 2 showed destruction of glomeruli and tubules. There was irregular lymphocytic infiltration at places. Such abnormalities are consistent with renal complications typically observed in diabetes due to oxidative stress and inflammatory response (Mateos *et al*., 2020). All necrotic alterations were absent in G 3 and in treatment groups G 4, G 5, and G 6 with methanolic extracts of *Turbinaria decurrens*. The group treated with methanolic extracts of *Turbinaria decurrens* 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 *Turbinaria decurrens* methanolic extracts. Similar observations have been documented in another study where both ethanolic and aqueous extracts of *Sargassum polycystum* significantly reduce renal degeneration in diabetic rats (Motshakeri *et al.*, 2014) with the improvement suggesting that brown algae bioactive compounds can contribute to renal protection, may mitigate hyperglycemia induced renal injury by reducing oxidative and inflammatory damage.

Groups	Kidney	Liver	Pancreas
G 1			
G 2			
G 3			
G 4			
G 5			

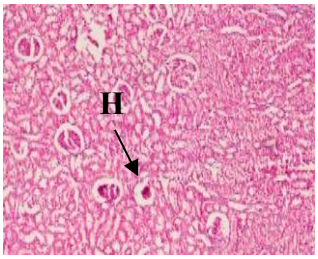
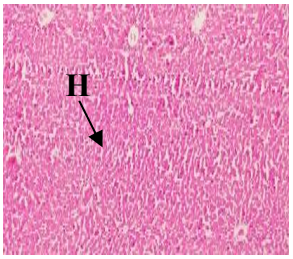
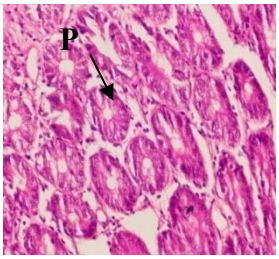
Groups	Kidney	Liver	Pancreas
G 6			

Figure 63: Histopathology of Kidney, Liver and Pancreas of G 1 (Control), G 2 (Diabetic control), G 3 (STZ-Metformin), G 4 (*TD* extract treated (100mg/kg), G 5 (*TD* 200mg/kg), and G 6 (*TD* 400mg/kg). Histological sections were visualized by staining with hematoxylin and eosin (H&E) observed under optical microscope (OLYMPUS Microscope) with 10x magnification.

Photomicrograph of kidney showed cortical sections C, showing the glomerulus and Bowman's capsule. R indicated a reduction in the cell types of the renal corpuscle, namely mesangial cells and capillary endothelial cells. O represented slight oedema, while L indicates lymphocytic infiltration in the kidney tubules.

Photomicrographs of liver showed intact hepatocytes and sinusoids (S) with a well-marked central vein (CV). Glycogen deposits (G) were visible along with a congested central vein (CCV).

Photomicrographs of pancreatic tissue showed IL -Islets of Langerhans and ID-interlobular duct, along with normal tubules. B represented degeneration and damage to β cells. T indicated disrupted tissue architecture with acinar cells not clearly visible. N showed nuclei becoming more prominent in the tissue. P represented normal acinar parenchyma of the pancreas along with ducts and islets of Langerhans. H indicated improvement in cellular architecture in the kidney and liver at medium and high doses of *TD* extract**.**

CHAPTER 5

SUMMARY & CONCLUSION

Marine macroalgae are gaining recognition as valuable reservoirs of bioactive compounds with significant therapeutic potential. In this study, particularly attention was given to the brown algae *Turbinaria decurrens,* a species known to harbor a wide array of secondary metabolites. Proper taxonomical and morphological identification was undertaken to ensure authenticity of the species, as reliable characterization is essential for linking metabolic profile to pharmacological activities. Phytochemical and biochemical analyses were carried out to evaluate the medicinal properties of *Turbinaria decurrens* with a specific focus on its antidiabetic potential. Comparative solvent extraction revealed that methanol yielded the highest percentage of extract, followed by acetone and aqueous extract. Subsequent analyses confirmed the presence of diverse classes of bioactivities including coumarins, glycosides, phenolics, tannins and terpenoids. The biological efficacy of these extracts was further assessed through a series of *in-vitro* antioxidant and antidiabetic assays. Methanolic extract consistently demonstrated superior antioxidant activity in ABTS, DPPH, FRAP, LPO as well as α-amylase and α-glucosidase inhibition assay. The antioxidant potential of the extracts can be attributed to their rich secondary metabolite content which effectively neutralizes reactive oxygen species and interrupts oxidative chain reactions. *In-vitro* cytotoxicity evaluation using the MTT assay on 3T3 cell lines confirmed the safety profile of the extracts and based on the preliminary investigation the methanolic extract of seaweed was chosen for in vivo studies in animal model. The acute toxicity profiling was carried out according to OECD guidelines (annexure 2b) revealed the safety of the extract upto 2000mg.kg body weight. The pharmacodynamic studies were evaluated in streptozotocin induced diabetic rat model. *Turbinaria decurrens* exhibited dose dependent ameliorative effects, including significant reduction in blood glucose levels, improvement in liver and lipid profiles and restoration of antioxidant status. Histopathological analysis provided additional evidence revealing marked improvement in kidney and pancreatic tissues of treated animals.

CONCLUSION: Overall, the results indicated that *Turbinaria decurrens* is a potentially marine source of medicinal bioactive chemicals with strong antidiabetic potential. The high phytochemical content of the methanolic extract was closely associated with its strong antioxidant and enzyme-inhibitory qualities. Its safety profile *in-vitro* and *in-vivo* along with notable improvements in biochemical and histopathological indices in diabetic wistar rats underscore its potential as a safe and effective preclinical antidiabetic drug.

These findings highlighted the need for more in-depth research, including studies to understand the underlying mechanisms and potential clinical trials to further validate the therapeutic promise of *Turbinaria decurrens* in managing diabetes.

5.1 RECOMMENDATIONS FOR FUTURE RESEARCH:

- Further investigations into molecular mechanisms by which *Turbinaria decurrens* exerts its antidiabetic and antioxidant effects are warranted. Understanding the specific signaling pathways associated with bioactive compounds could elucidate its therapeutic mechanisms.
- Further studies should aim to isolate and characterize specific phytochemical compounds of *Turbinaria decurrens* to assess their individual contribution for antioxidant and antidiabetic activity.
- To establish the efficacy and safety, bioassay guided fractionation of methanolic extract may be undertaken to isolate and characterize principal active constituent thereby facilitating futuristic development of new drug candidate with antidiabetic potential.

5.2 FUTURE SCOPE OF THE STUDY: The present investigation underscores the therapeutic promise of *Turbinaria decurrens* as a natural intervention against diabetes and associated oxidative stress. Building on these findings, several directions for future research can be recommended:

- Further research should aim to unravel the molecular basis of the antidiabetic action of *Turbinaria decurrens.* Particular attention could be given to its influence on insulin signaling, oxidative stress related pathway and gene expression involved in glucose homeostasis.

- While preliminary findings suggest a favorable safety margin, extended studies are necessary to evaluate chronic exposure, potential organ specific effects, and sustained pharmacological activity in diabetic models.
- To establish therapeutic relevance, controlled clinical studies are required. These investigations would validate its efficacy in human subjects and may also identify its role in mitigating diabetes associated complications such as nephropathy or cardiovascular risks.
- Designing stable and standardized formulations, including dietary supplements or functional foods enriched with *Turbinaria decurrens* extracts could facilitate its integration into preventive and therapeutic regimens.
- Investigating the potential of *Turbinaria decurrens* in combination with conventional antidiabetic drugs or other natural compounds may uncover synergistic interactions offering more effective treatment strategies with reduced side effects.

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ANNEXURE 1

CERTIFICATE OF CONFERENCES AND POSTER PRESENTATION

Certificate 1

KHALSA COLLEGE AMRITSAR

AN AUTONOMOUS COLLEGE

28th

PUNJAB SCIENCE CONGRESS

National Conference

on

Current Trends in Science and Technology

UNDER THE AEGIS OF

PUNJAB ACADEMY OF SCIENCES, PATIALA

CERTIFICATE

This is to certify that Dr./Mr./Ms. Juhi Kataria of Dept. of Medical LPU Jalandhar has participated/presented paper (oral/poster) entitled "Extraction Characterization ..... Seaweed Turbinaria decurrens" in 28th Punjab Science Congress and National Conference on "Current Trends in Science and Technology" from February 7- 9th 2025 organized by Faculty of Sciences, Khalsa College Amritsar, Punjab.

Dr. Arvinder Kaur Kahlon
Principal & Chief Co-ordinator

Dr. Taminder Singh
DAA & Co-ordinator

Dr. Rajbir Singh Sohi
Organizing Secretary

Certificate of participation of Oral presentation on Extraction, Characterization in vitro antioxidant activity of seaweed *Turbinaria decurrens*. 28th Punjab Science Congress National Conference on Current Trends in Science and Technology organized by Faculty of Sciences, Khalsa College Amritsar, Punjab 7th -9th February 2025.

Certificate 2

Certificate of participation of Oral presentation on Genus *Turbinaria* Coral diversity, Resilience and conservation in the face of global environmental change. 12th International Advanced Diagnostic Techniques Conference organized by Allied Health Sciences Department Chitkara School of Health Sciences, Chitkara University, Punjab 15th -16th March 2024.

Certificate 3

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

Certificate of Achievement

This is to certify that Prof./Dr./Mr./Ms. Jushi Kataria..... of ...Lovely Professional Uni..has presented Oral/Poster entitled. Turbinaria treasures: Unveiling the Wonders of Coral Div..In **International Conference on Recent Trends in Biomedical Sciences (RTBS-2023)** organized by Department of Medical Laboratory Sciences from 6th-7th October 2023 at Lovely Professional University, Punjab, India.

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

Prof (Dr.) Monica Gulati
Chairperson
RTBS-2023

Certificate of participation of Poster presentation on *Turbinaria* treasures: Unveiling the wonders of Coral diversity. International conference on Recent Trends in Biomedical Sciences (RTBS 2023) organized by Department of Medical Laboratory Sciences on 6th -7th October 2023.

Certificate 4

CERTIFICATE

This is to certify that Prof./Dr./Mr./Ms. JUHI KATARIA of. ~~Lovely Professional~~ University has participated in Poster Presentation/~~Oral Presentation~~ on the topic entitled. ~~Taxonomic, morphological identification and extraction of brown seaweed~~ Turbinaria decurrens in 2nd International Conference on Plant Physiology and Biotechnology (ICPPB) held from 20-21 April 2023 organized by School of Bio-engineering and Biosciences, under the aegis of Lovely Professional University, Punjab.

Dr. Vijay Kumar
Organizing Secretary

Dr. Umesh Goutam
Co-Convener

Dr. Neeta Raj Sharma
Convener

Certificate of Participation of poster presentation on Taxonomical, morphological and extraction of brown seaweed *Turbinaria decurrens.* 2nd International conference on Plant Physiology and Biotechnology (ICPPB) organized by School of Bio-engineering and Biosciences LPU Punjab on 20th – 21st April 2023.

Certificate 5

Certificate of Poster presentation on Seaweed *Turbinaria decurrens* and its importance in National Seminar World of Millets in the Era of Climate Change organized by Botanical and Environmental Science Society, Post Graduate Department of Botany, Khalsa College Amritsar 1st – 3rd March 2023.

ANNEXURE 2:

RESEARCH & REVIEW ARTICLE PUBLICATION

RESEARCH ARTICLE 1

Journal of Neonatal Surgery
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OPEN ACCESS

Evaluation of Phytochemical Constituents, Antioxidant & Anti-Diabetic Activity of Seaweed Turbinaria Decurrens Collected from Gulf of Mannar, Tamil Nadu, India

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ABSTRACT

The genus *Turbinaria* stands out as a prominent and widely distributed member of the brown algae. This species is characterised by distinct morphology, ecological importance, and biochemical properties. In the present study, taxonomical, molecular identification along with antioxidant and anti-diabetic activity of the different extracts (acetone, aqueous & methanol) of *T.decurrens* was determined. The preliminary phytochemical components were investigated qualitatively and quantitatively by using standard methods and confirmed the presence of variety of phytoactive components such as coumarins, glycosides, phenols tannins, terpenoids and saponins. The collected seaweed sample was identified by molecular approaches using QIAGEN DNA isolation kit and the BLAST program at the NCBI website that showed se quences similarities with existing seaweed rbcL gene sequences up to 99.21%. "The antioxidant activity of three crude extracts was evaluated by various antioxidant assays viz DPPH and ABTS." The findings revealed potent scavenging activity of ABTS, with a minimum inhibitory concentration (IC_{50}) of 1.06mg/ml for the methanolic extract while the DPPH assay indicated an IC_{50} value 1.56mg/ml. The investigation into the antidiabetic properties of seaweed extract revealed that the methanolic extract of *T.decurrens* exhibited the most significant inhibitory activity against α-amylase and α-glucosidase enzymes with IC_{50} value of 1.8 mg/ml and 1.40mg/ml respectively. The presence of phytochemical metabolites contributes to both antioxidant activity and anti-diabetic activities. These results indicate that the extract of *T.decurrens* serve as a natural source of antioxidants and anti-diabetics compounds possessing the potential to address a wide array of serious health conditions.

1. INTRODUCTION

Seaweeds have garnered significant attention recently due to their high concentrations of physiologically active compounds. They can be utilised in various applications within the food, cosmetics, pharmaceutical, and environmental industries [1]. Seaweeds hold significant potential for medication research and nutritional supplements due to their abundance of bioactive components, including polysaccharides, polyphenols, pigments, and peptides, which exhibit anti-inflammatory, antiviral, antioxidant, and anticancer activities. Seaweeds are primarily classified into three types based on their pigment composition: Green Algae (Chlorophyta), Red Algae (Rhodophyta), and Brown Algae (Phaeophyceae). In literature, numerous chemicals like Agar, Carrageenan, Fucoidan, Laminaran, and Phlorotannins have been extracted from seaweeds, acknowledged for their distinctive properties and interactions with biological systems that may confer health advantages [4-5].

The species T. decurrens is notable and extensively spread among brown algae. The genus Turbinaria is classified within the family Sargassaceae and the class Phaeophyceae. T. decurrens is a common component of both stony and coral reefs, suggesting its significant role in the ecosystem [7]. Compounds extracted from T. decurrens have been observed to prevent aberrant cell proliferation, have antipyretic activities, reduce inflammation, enhance immunological function, and assist in hyperglycemia management [8]. Hyperglycemia is a defining characteristic of diabetes, a metabolic disorder necessitating ongoing medical management. This can arise from several factors, including tissue resistance to insulin, diminished insulin activity, or complete insulin shortage. Diabetes Mellitus (DM) is classified into Type 1 diabetes mellitus, often referred to as insulin-dependent diabetes mellitus (IDDM), which is characterised by the absence of insulin secretion due to damage to pancreatic β cells. "Furthermore, it accounts for roughly 10% of all diabetes cases. Conversely, Type 2 diabetes mellitus, also known as non-insulin dependent diabetes mellitus (NIDDM), impacts approximately 90% of individuals and is characterised by tissue insensitivity to insulin activity." Hyperglycemia is thought to substantially contribute to oxidative stress. It initiates the auto-oxidation of glucose, leading to the excessive generation of free radicals that induce cellular

RESEARCH ARTICLE 2

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RESEARCH ARTICLE

Acute toxicity and cytotoxicity assessment of *Turbinaria decurrens* methanolic extract in Wistar rats

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ABSTRACT:

Seaweed *Turbinaria decurrens* (*T. decurrens*) has attracted considerable attention in pharmaceutical research due to its rich composition of bioactive constituents, including polysaccharides, polyphenols, pigments, proteins, fatty acids, and various secondary metabolites with health promoting properties. Notwithstanding the therapeutic benefits of *T. decurrens*, evidence about its detrimental effects remained few. This work focused on the toxicity of the methanolic extract of *T. decurrens* using Wistar rats' models. The current investigation involved the extraction of seaweed compounds utilizing three distinct solvents: Acetone, Aqueous, and Methanol. These extracts were subjected to evaluate the MTT cytotoxicity assay (3-4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide on 3T3 fibroblast cell lines. Based on the preliminary phytochemical screening and antioxidant profiling, methanolic extract was selected for further toxicological evaluation. An acute toxicity investigation was performed on female Wistar rats, providing a single dosage of 50 mg/kg, 300 mg/kg, and 2000 mg/kg of body weight of methanolic extract. The animals were observed for 14 consecutive days for behavioural changes and signs of toxicity. Upon completion of the trial, body weights measurements were taken followed by haematological and biochemical analyses. The animals were sacrificed at the end of the trial, and the essential organs, liver and kidney, were removed to identify any potential histological abnormalities. The data demonstrated no fatalities throughout the trial. No notable alterations were seen in general behaviour, body weight, or biochemical and haematological markers among the groups administered 50 mg/kg, 300 mg/kg, and 2000 mg/kg of methanolic extract of *T. decurrens*. Histopathological analysis indicated no abnormalities in the morphology of the liver and kidney in the groups treated with 50 mg and 300 mg/kg; however, mild lymphocyte infiltration was observed in the kidney of the group administered 2000 mg/kg body weight of methanolic extract of *T. decurrens*. The study revealed that the ingestion of *T. decurrens* at doses up to 2000 mg/kg is safe and does not result in any structural harm to organs. Consequently, the normal blood profile, together with biochemical and histological analyses, indicated non-toxic characteristics and safe use of *T. decurrens*.

KEYWORDS: Cytotoxicity, Acute toxicity, Behavioral changes, Turbinaria decurrens, seaweeds, pharmaceutical drugs.

INTRODUCTION:

Seaweeds have gained importance in pharmaceutical research due to their extensive range of bioactive compounds with potential therapeutic applications. Specifically, brown algae are rich in various natural substances, including polysaccharides, proteins, polyphenols, pigments, fatty acids, and secondary metabolites with various biological activities.[1] Significant attention from researchers has been given to the seaweed extracts because compounds present in the extracts such as flavonoids, alkaloids, saponins, carbohydrates and terpenoids[2] showed apparent antioxidant[3], anti-inflammatory, antiviral, antibacterial,

REVIEW ARTICLE 1

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Review
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Turbinaria: Exploring the Untapped Potential of Bioactive Compounds from Nature's Marine Brown Algae

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Abstract: The study of seaweeds has garnered significant scientific attention for over seven decades, revealing their abundant composition of enzymes, polysaccharides, trace minerals, essential vitamins, and a variety of bioactive compounds. These compounds possess certain health-promoting activities, including antiviral, antibacterial, and antioxidant effects, which contribute to their potential therapeutic value. Among Marine algae, the genus *Turbinaria* stands out for its robust antioxidant, antidiabetic, and anticancer activities, as well as a range of other pharmacological activities. This review will elucidate and emphasize the roles of algae, notably *Turbinaria*, in drug development, their ability to substitute for synthetic drugs, and provide insights into the treatment of anticancer and other neurological disorders. To facilitate effective use of seaweeds, further research will be required to understand the mechanisms of action of these bioactive compounds, to enable their use in certain aspects of public healthcare and commercial applications.

Keywords: *Turbinaria*; bioactive compounds; seaweed; drug development; pharmaceutical applications; biological activities.

1. Introduction