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Phytochemical Profiling and Investigating of Anti-Diabetic Properties of *Asparagopsis taxiformis* Collected from the Bay of Bengal Bangladesh

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Abstract

Diabetes mellitus (DM) is a global health issue due to its prevalence and catastrophic health effects. Synthetic hypoglycemic agents can treat diabetes, but they have side effects. Therefore, natural remedies for diabetes are now gaining popularity. Marine benthic algae are rich in phytochemicals and other bioactive compounds. Inhibition of carbohydrate hydrolyzing enzymes *in-vitro* and lower blood glucose levels *in-vivo* during fasting and postprandial testing imply seaweed extracts and their bioactive ingredients may treat diabetes. This study investigated the phytochemical properties of *Asparagopsis taxiformis* from the southern part of St. Martin Island in Bangladesh and examined the anti-diabetic activity of its 50% ethanolic extracts *in-vitro* and *in-vivo*. After establishing traditional phytochemical presence, total phenolic and flavonoid content was estimated, and an *in-vitro* alpha-amylase inhibitory activity test was performed. *In-vitro* anti-diabetic investigation shows that 50% ethanolic extract of *Asparagopsis taxiformis* reduces diabetes less than acarbose. *In-vivo* anti-diabetic tests also showed similar results compared to the control group when their blood glucose level was measured. After 14 days of treatment with the 50% ethanolic extract of *Asparagopsis taxiformis*, the treated group of mice didn't show any considerable lowering activity when their body weight, lipid profile, kidney function, and liver function (SGPT, SGOT) were compared to the control group. Though *Asparagopsis taxiformis* has manifold benefits, 50% ethanolic extract of this alga didn't show any antidiabetic properties suggesting more studies in different solvents are required to evaluate the antidiabetic properties.

Keywords: *Asparagopsis taxiformis*, Phytochemical, Antidiabetic, *in Vitro*, *In Vivo*.

INTRODUCTION

Diabetes mellitus is a persistent metabolic disorder caused by insufficient insulin levels, leading to elevated blood glucose levels. This condition is characterized by hyperglycemia, disrupted carbohydrate, protein, and lipid metabolism, and an augmented susceptibility to vascular complications (Verma *et al.*, 2010). Diabetes mellitus can be classified into two primary categories, namely type 1 and type 2, based on its etiology. Type 1 diabetes is characterized by an autoimmune response that leads to the destruction of β-cells, whereas type 2 diabetes is primarily driven by insulin resistance. An alteration in the process of insulin production and its effects gives rise to irregularities in the metabolic pathways of proteins, fats, and carbohydrates. This, in turn, leads to elevated levels of glucose in the blood (hyperglycemia), increased levels of lipids in the blood (hyperlipidemia), inflammation, and oxidative stress. These factors are believed to have a significant role in developing and advancing difficulties associated with diabetes (Hameed *et al.*, 2015; Ibrahim *et al.*, 2022). According to the 2022 diabetic study report, the global prevalence of diabetes is estimated to be around 357 million individuals across all age groups. This number is projected to increase significantly to approximately 783 million individuals by the year 2045 (Gull *et al.*, 2023). Based on projections from the International Diabetes Federation (IDF), there are currently 7.1 million people living with diabetes in Bangladesh, with an equal number of undiagnosed cases. By 2025, this figure is expected to double. The standard

therapeutic approach for diabetes mellitus (DM) encompasses the administration of insulin through injections, as well as the utilization of several anti-diabetic medications, including sulfonylureas, metformin, glinides, biguanides, and acarbose (Ma, 2014; Glossmann and Lutz, 2019). Although many anti-diabetic medicines have demonstrated efficacy in reducing and controlling blood glucose levels, a significant proportion of them are associated with unfavorable side effects such as gastrointestinal disorders, anemia, renal failure, weight gain, and hypoglycemia. Hence, the pursuit of novel natural remedies exhibiting enhanced efficacy and improved safety profiles assumes paramount importance in the quest for the development of innovative anti-diabetic therapeutics (Dinda and Dinda, 2022).

Seaweed constitutes the ocean's most ubiquitous resource. In addition to minerals and vitamins, seaweed comprises polysaccharides, proteins, amino acids, lipids, and peptides. In the cosmetic and pharmaceutical industries, polyphenols derived from seaweed were utilized for their antioxidant, anti-radiation, antibiotic, anti-inflammatory, hypoallergenic, antimicrobial, and anti-diabetic properties. Algae exhibit rapid adaptation in response to the extreme environmental conditions (alterations in salinity, temperature, nutrients, and ultraviolet radiation) to which they are exposed, as well as the complex habitats in which they exist. To thrive in these conditions, algae undergo a growth cycle in which they generate an extensive array of bioactive compounds. These compounds consist of minerals, dietary fibers, essential amino acids derived from high-quality proteins, and chemical compounds that possess a wide range of relevant biological activities and are unique to algae (Mellouk *et al.*, 2017).

Seaweed polyphenol extracts, such as those found in Alaria, Ascophyllum, Padina, and Palmaria, inhibit the activity of α-amylase and α-glucosidase, thereby potentially reducing glucose levels in the blood. Conversely, seaweed is abundant in antioxidants, which may provide diabetes patients with beneficial properties (Husni, 2018). *Gracilaria verrucosa* (GV), *Undaria pinnatifida sporophyll* (UPS), and *Codium fragile* (CF) are examples of seaweeds that exhibit anti-diabetic properties. The inhibitory effects of water extracts derived from these seaweeds on the absorption of excess glucose and its utilization in alternative cellular pathways have been well-documented. Consequently, the surplus glucose is prevented from accessing the bloodstream. It has been reported that several additional red, green, and brown seaweeds exhibit anti-diabetic properties (Joy Lindsey *et al.*, 2021). *Asparagopsis taxiformis*, commonly known as red seaweed, possesses remarkable medicinal properties and has garnered significant attention for its potential commercial applications. Rich in bioactive compounds, this seaweed exhibits anti-inflammatory, antioxidant, and antimicrobial properties, making it a promising candidate for pharmaceutical and nutraceutical development. Additionally, *Asparagopsis taxiformis* is renowned for its unique ability to produce halogenated secondary metabolites, particularly bromoform and dibromomethane, which have shown promise in inhibiting methane production in ruminant animals, thereby contributing to efforts in mitigating greenhouse gas emissions from livestock. The commercial potential of *Asparagopsis taxiformis* extends to the cosmetic industry, where its extracts are explored for skincare formulations due to their anti-aging and skin-renewing properties. With ongoing research and sustainable cultivation practices, *Asparagopsis taxiformis* stands at the forefront of harnessing marine resources for both medicinal and commercial purposes (Paul, De Nys and Steinberg, 2006; Kinley *et al.*, 2016).

There has been no investigation into the anti-diabetic properties of extracts derived from the red seaweed *Asparagopsis taxiformis*. This study aims to determine the anti-diabetic properties of 50% ethanolic extract of *Asparagopsis taxiformis*, collected from the Bay of Bengal, Bangladesh.

MATERIALS AND METHOD

Sample Collection and Validation

Seaweed samples were collected from the on the southern side of St. Martin's Island (Chera Dwip), Bangladesh. The samples were first cleaned using seawater to eliminate sand particles, epiphytes, and other contaminants. To remove residual salt, the samples were washed with distilled water. The samples were validated as *Asparagopsis taxiformis* based on visual appearance, collection site, and seasonal availability data (Islam *et al.*, 2020). Collected *Asparagopsis taxiformis* samples were then sun dried and kept in airtight containers for further study.

Sample Processing

The sun-dried samples were later further dried at 45° C to achieve a crisp texture using a dryer (Model: JSON 030S JSR, Korea). The crisped samples were then ground into a fine powder with a mortar and pestle. 15 grams of weighed powdered sample was then soaked in 150 mL 50% ethanol. The soaking process lasted for 5 days, during which the flask was kept at room temperature (37° C) and subjected to agitation at a speed of 150 rpm using a shaking incubator (Model: JSSI-070C, JSR, Korea). The initial concentration of the extract was established at 100 mg/ml (dry weight/volume) (Chowdhury *et al.*, 2023). The extracts were subsequently passed through Double Rings of 11.0 cm filter paper (Qualitative, 102), and the resulting filtrate was preserved at a temperature of 4° C for subsequent analysis.

Phytochemical Screening

A phytochemical screening was conducted utilizing appropriate analytical reagents to identify the

presence of bioactive phytoconstituents in the extracts. The identification of phytoconstituents was conducted using established methodologies (Richardson and Harborne, 1990; Khoo *et al.*, 2018).

UV-Visible Spectroscopy Analysis

Spectrophotometric analysis of the *Asparagopsis taxiformis* extract was conducted using a Shimadzu UV 1900 UV-Visible spectrophotometer with a slit width of 1.0 nm, while maintaining room temperature. The proximate analysis involved the examination of the extract using visible and UV light within a wavelength range of 190 to 1100nm. The sample was centrifuged at a speed of 3000 rpm for 10 minutes following filtration through Whatman No. 1 filter paper. This process was conducted to prepare the extract for subsequent analysis using a UV-VIS spectrophotometer. The sample was diluted at a ratio of 1:10 using a 50% ethanol solution as the solvent for the sample. The baseline was adjusted using identical solvents, particularly a 50% ethanol solution. The UV-VIS spectrophotometer was allowed to warm up for 30 minutes before commencing the investigation (Chowdhury *et al.*, 2023).

Determination of Total Phenolic Contents (TPC)

Spectrophotometric analysis was used to calculate the phenolic content of algal extract (Singleton, Orthofer and Lamuela-Raventós, 1999). The evaluation used a 50% ethanolic solution liquid extract at a concentration of 100 mg/ml. Mixing 0.5 ml of a 50% ethanolic solution of the extract with 2.5 ml of 10% Folin-Ciocalteu's reagent (FCR) in water and 2.5 ml of 7.5% $NaHCO_3$ yielded the reaction combination. Simultaneously, a blank comprising 0.5 ml of 50% ethanol, 2.5 ml of 10% Folin-Ciocalteu's reagent (FCR) in water, and 2.5 ml of 7.5% $NaHCO_3$ was produced. After that, the samples spent an additional 45 minutes in an incubator at 45° C. Using a spectrophotometer, the absorbance was measured at a wavelength (λ_{max}) of 765 nanometers (nm). Triplicate samples were generated for each analysis, and the average absorbance was calculated. The gallic acid standard solution was analyzed similarly to determine the calibration curve. Based on the measured absorbance, the concentration (2, 4, 8, 16, 32, 62.5, 125, 250, 500) of phenolics was read (µg/ml) from the calibration curve; subsequently, the content of phenolics in algal extracts was represented in terms of gallic acid equivalent (mg of GA/ml of extract).

Determination of Total Flavonoid Contents (TFC)

The overall flavonoid concentration was quantified using a colorimetric technique (Fattahi *et al.*, 2014). A volume of 100 µL of an algal 50% ethanolic extract was introduced into 4 mL of distilled water. Subsequently, a volume of 0.3 ml of a 5% sodium nitrite ($NaNO_3$) solution was introduced. Following a time interval of 5 minutes, a volume of 0.3 milliliters of a solution containing 10% aluminium chloride ($AlCl_3$) was introduced. Within a time, frame of 6 minutes, a volume of 2 milliliters of a sodium hydroxide (NaOH) solution with a concentration of 1 molar was introduced into the combination. Promptly, the concoction was diluted by incorporating 3.3 milliliters of distilled water, followed by thorough mixing. The absorbance measurement was conducted at a wavelength of 510 nm compared to a blank sample. Concurrently, a solution consisting of the identical reagent was employed, except that it contained 50% ethanol instead of a 50% ethanolic liquid extract. Catechin was employed as the reference substance across several concentrations (2, 4, 8, 16, 32, 62.5, 125, 250, 500, 1000) to establish the calibration curve, with the concentrations expressed in micrograms per milliliter (µg/ml). The quantification of the total flavonoid content in the extract was represented as milligrams of catechin equivalents per ml of the sample (mg/ml).

In-Vitro Anti-Diabetic (α-amylase inhibition) Assay

The experiment was carried out by the method described by Mitra *et al.*, (Tamil *et al.*, 2010). The substrate solution combined 6 mg of starch with 0.6 mL of a 0.01 M $CaCl_2$ (pH 6.9) and 0.5 M Tris-HCl buffer (pH 6.9) solution. The substrate solution was pipetted into test tubes, boiled (for 5 minutes), and preincubated (for 5 minutes) at 37 degrees Celsius. Different algal extract and acarbose concentrations were made by dissolving them in DMSO. The algal extract or acarbose solution (0.6 mL) at varied concentrations was added to the test tube containing the substrate solution, and later on, the porcine pancreatic alpha amylase (0.3 mL in Tris-HCl buffer (2 units/ mL)) was added. After 10 minutes of incubation at 37° C, each test tube was topped off with 1.5 mL of 50% acetic acid to finish the process. The supernatant optic density measured at 595 nm after centrifugation (at 3000 rpm for 5 minutes at 4° C). This experiment employed acarbose, an alpha-amylase inhibitor, as a positive control. The tests were conducted in triplicates.

The extract's inhibitory activity against alpha-amylase was determined using the following formula:
Inhibitory activity = $[(X_A - X_B)/X_A] \times 100$.
X_A is the absorbance of the control (100% enzyme activity) and X_B is the absorbance of the sample.

In-Vivo Anti-Diabetic Assay
Animals

Female Swiss albino mice weighing between 20 and 30 grams were obtained from the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr, b). The animals were housed under controlled laboratory settings, with a relative humidity of 55±5%, a room temperature of 25±3°C, and a 12-hour light-dark cycle. They were provided with available access to both water and food. Before the tests, a one-week acclimatization period was implemented for all animals to mitigate the potential impact of stress-induced physiological, cardiovascular, immunological, central nervous system, and endocrine system abnormalities

resulting from transportation (Obernier and Baldwin, 2006). The ethical clearance for employing a mice model in this research was received from the appropriate authority at Bangabandhu Sheikh Mujibur Rahman Maritime University, located in Dhaka, Bangladesh.

Induction of Experimental Diabetes

The experiment involved utilizing female Swiss albino mice, which were subjected to an overnight fasting period. The mice's weight and fasting blood glucose level were carefully measured and documented during this time. Alloxan was individually weighed for each animal based on their respective body weights. Subsequently, it was dissolved in 0.9% (w/v) normal saline immediately before injection. The alloxan solution was then administered intraperitoneal (IP) route to overnight fasted mice at a dosage of 180 mg/kg/body weight to induce experimental diabetes in the mice. The animals were provided with food and drink 30 minutes after alloxan administration (Nagappa *et al.*, 2003). To mitigate the occurrence of hypoglycemia shock and fatalities during the hypoglycemic phase, a solution consisting of 10% glucose in tap water was administered through a water bottle over 24 hours. The plasma glucose level of each animal was evaluated for four days following the administration of alloxan. Animals with a fasting blood glucose level exceeding 200 mg/dL were selected for inclusion in the study. The blood samples were obtained from the caudal region of the mice (Gidado, Ameh and Atawodi, 2005).

Experimental Design

The animals were sorted into six groups using a random allocation method, each consisting of five mice in the following manner:

Group I: Normal untreated mice given normal diet. (Normal Control)

Group II: Diabetic untreated mice given distilled water (10 ml/kg of body weight).

Group III: Diabetic mice given seaweed extract at a dose of 100 mg/kg body weight.

Group IV: Diabetic mice given seaweed extract at a dose of 50 mg/kg body weight.

Group V: Diabetic mice given (standard) glibenclamide at a dose of 0.66mg/kg body weight.

Group VI: Diabetic mice given 50% ethanol as a negative control.

The mice in each group were subjected to daily administration for 14 days. Blood glucose levels (BGL) were assessed by extracting blood from the tail of each mouse. During the acute studies, specimens were gathered at three time points: 0, 2, and 4 hours after administration. During the chronic trials, blood glucose levels (BGL) were assessed every week for a period of two weeks.

Body Weight Determination

The body weights of all groups of mice were recorded before the initiation of therapy and daily throughout the duration of the treatment period. The body weight of the experimental mice was measured using a suitably calibrated electronic balance.

Biochemical Parameter Analysis

On the 14th of therapy, after an overnight period of fasting, the mice were sacrificed using chloroform as an anesthetic. The blood was obtained from the heart using a puncture, and the serum was subsequently separated by centrifugation at a speed of 3000 revolutions per minute (rpm) for 10 minutes, resulting in the isolation of the supernatant fluid. Subsequently, the fluid was transferred to a separate microcentrifuge tube to conduct biochemical experiments. Diabetes has a unique impact on the lipid profile and liver function. The renal function in individuals with diabetes is promptly impacted, leading to the potential development of renal failure in the long term (Joy Lindsey *et al.*, 2021). Hence, the serum was obtained to conduct a lipid profile analysis, which includes measurements of triglyceride (TG), total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL). Additionally, kidney function was assessed by measuring creatinine, while liver function tests were performed to measure levels of glutamic-oxalacetic transaminase (SGOT) and glutamic-pyruvic transaminase (SGPT). These analyses were carried out with the assistance of Lions Eye Institute & General Hospital professionals.

Statistical Analysis of the Result

The data from the study was analyzed with Microsoft Excel 2013. Mean and standard error of the mean (±SEM) were then used to report the findings. Differences between means of all parameters were done using ANOVA. The cause of the significant differences was then identified using Tukey's post hoc testing for multiple comparisons. The statistical cutoff for significance was set at $P<0.05$.

RESULTS

Phytochemical Analysis

Seaweed has significant phytoconstituents that contribute to a range of biological activities.

The initial evaluation of the 50% ethanolic extract of *Asparagopsis taxiformis* revealed the existence of a wide range of phytochemical components. **Table 1** presents the phytochemical elements found in the extract of *Asparagopsis taxiformis*. The analysis revealed that the primary phytoconstituents present in the extract of *Asparagopsis taxiformis* were flavonoids, steroids, phenols, saponins, carbohydrates, amino acids, glycosides, terpenoids, and quinones.

Table 1: Phytochemical screening of *Asparagopsis taxiformis* extracts obtained by 50% ethanol

Name of The Compound	Test Name	Present (+)/Absence (-)
Alkaloid	Mayer's Test	-
	Wagner's Test	-
Flavonoid	Alkaline Reagent Test	+
	10% Lead Acetate Test	-
	Sulfuric Acid Test	-
Steroid	Salkowski Test	+
Phenol	Ferric Chloride Test	-
	10% Lead Acetate Test	+++
Tannin	5 % Ferric Chloride Test	-
Saponin	Foam test	++
Carbohydrate	Molisch Test	+
Amino Acid	Ninhydrin Test	+
Glycosides	Salkowski Test	+
Terpenoid	Alkaline Reagent Test	++
Coumarin	Alkaline Reagent Test	-
Quinone	Acid Test (H_2SO_4)	+
	Acid Test (HCl)	+

Note: (-): Not detectable; (+): Low quantities; (++): Moderate quantities; (+++): High quantities

UV-Visible Spectroscopy Analysis

50% ethanolic extract *A. taxiformis's* was subjected to UV-VIS examination (at wavelengths between 190 and 1100 nm) to determine the chromophores, aromatic rings, and chemical compounds having σ-bonds, π bonds, lone pair electrons, and many other features. The profile showed peaks at 196.50 nm with absorption values of 1.034. *Asparagopsis taxiformis* extract's absorption spectrum is displayed in **Table 2;** the UV-VIS spectra reveal that this extract is especially transparent in the wavelength range of 190 to 1100 nm. However, the application of UV-VIS spectrophotometry in studying complex media is limited by the inherent difficulties of assigning the absorption peaks to any specific system constituents. It is necessary to corroborate UV-VIS results using additional analytical methods, including GC/MS, to facilitate extract characterization and ingredient identification.

Table 2: Compound detection of *Asparagopsis taxiformis* extract

Compound detection of *Asparagopsis taxiformis* extract on UV-Vis Spectrophotometer				
Wavelength range	Wavelength range Scanning Speed	Sample Interval	Detection (nm)	Absorption
190-1100nm	slow	0.5	196.50	1.034

Total Phenolic Contents (TPC) and Total Flavonoid Contents (TFC)

Flavonoids encompass a diverse array of naturally occurring compounds, characterized by their phenolic structures, and are predominantly present in fruits, vegetables, nuts, tea, and herbs. Previous research has indicated that most dietary flavonoids have diverse medicinal properties, such as antidiabetic potential. Rutin, a plant pigment has the ability to hinder carbohydrate absorption through the inhibition of the α-glucosidase enzyme. Similarly, kaempferol has been found to augment glucose uptake, whereas luteolin has been shown to restrict lipid synthesis (Vinayagam and Xu, 2015; Al-Ishaq *et al.*, 2019). Hence, the primary emphasis of this work was directed towards investigating flavonoids, which have been proposed as bioactive constituents. To conduct an initial assessment of the bioactive components, present in the algal extract, the total phenolic content (TPC) and total flavonoid content (TFC) of the extract were determined using colorimetric assays. The total phenolic content (TPC) value was determined using a gallic acid calibration curve with the equation y = 0.0073x - 0.0155 and a coefficient of determination (R2) of 0.9998 [Figure 1 (a)]. The TPC value was expressed as milligram gallic acid equivalent per milliliter of extract (mg GAE/ml extract). Similarly, the total flavonoid content (TFC) value was obtained using a catechin calibration curve with the equation y = 0.0004x + 0.0046 and an R^2 value of 0.9983 [Figure 1 (b)]. The TFC value was expressed as milligram catechin equivalent per milliliter of extract (mg CE/ml extract).

According to the findings shown in Table 3, it was observed that the quantities of both total phenolic content (TPC) and total flavonoid content (TFC) were detected in the 50% ethanolic extract of *Asparagopsis taxiformis*. The primary components accountable for the diverse anti-diabetic effects shown in medicinal plants and diets are phenolics and flavonoids (Ong and Khoo, 2000). Elevated levels of total phenolic content (TPC) and total flavonoid content (TFC) serve as potential indicators of the presence of therapeutic properties within the extracts (Babbar *et al.*, 2014). Therefore, it was expected that the 50% ethanolic extract of *Asparagopsis taxiformis*, containing both total phenolic

content (TPC) and total flavonoid content (TFC), would have anti-diabetic properties.

Table 3: TPC and TFC of 50% ethanolic extract of *Asparagopsis taxiformis*

50% Ethanolic Extract Concentration	TPC mg GAE/ml Extract	TFC mg CE/ml Extract
100 mg/ml	0.03	0.006

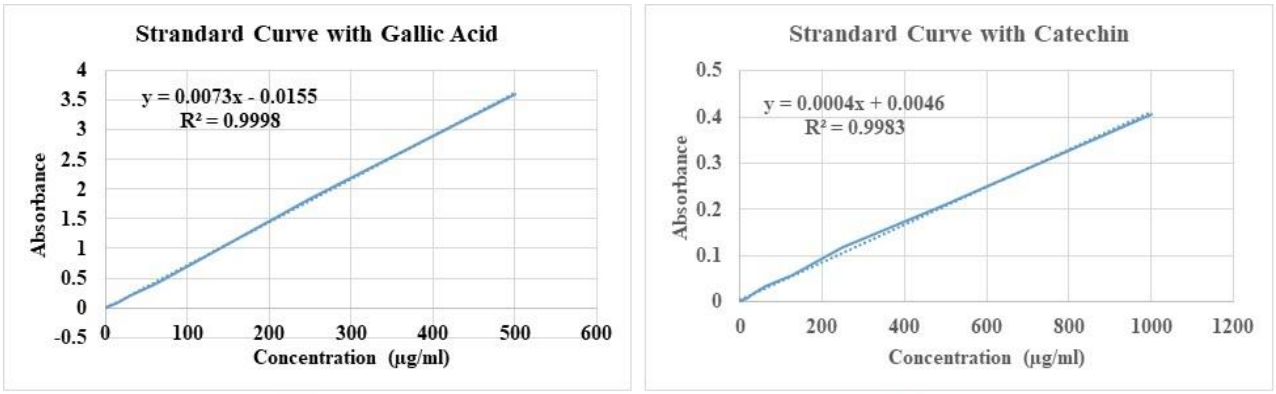

Figure 1: Standard Curve for the Measurement of TPC (a) and TFC (b)

Asparagopsis taxiformis In –Vitro Inhibition Potential on Alpha-Amylase Enzyme

Preventing glucose absorption is widely recognized approach in treating diabetes. The prevention of postprandial hyperglycemia is achieved by inhibiting the activity of digestive enzymes responsible for the hydrolysis of polysaccharides into smaller, more readily absorbable fragments. Alpha-amylase represents one of the enzymes in question (Qurtam *et al.*, 2021). Alpha-amylase is widely recognized as a crucial enzyme in digestion (Hsiu, Fischer and Stein, 1964), owing to its pivotal role in the hydrolysis of polysaccharides. Saliva and pancreatic juice are well recognized as the predominant sources where it is commonly detected. One potential strategy for mitigating the rise in blood glucose levels after a meal is to specifically target and decrease the activity of this particular enzyme (Alqahtani *et al.*, 2020). Figure 2 illustrates the inhibitory effects of acarbose and *Asparagopsis taxiformis* on alpha-amylase activity. The inhibitory potential of acarbose (the positive control) was 11.06±2.59%, while that of *Asparagopsis taxiformis* was -12.13±3.53%, according to the estimated percent inhibition. *Asparagopsis taxiformis* had little or no effect on inhibiting the alpha-amylase enzyme compared to acarbose.

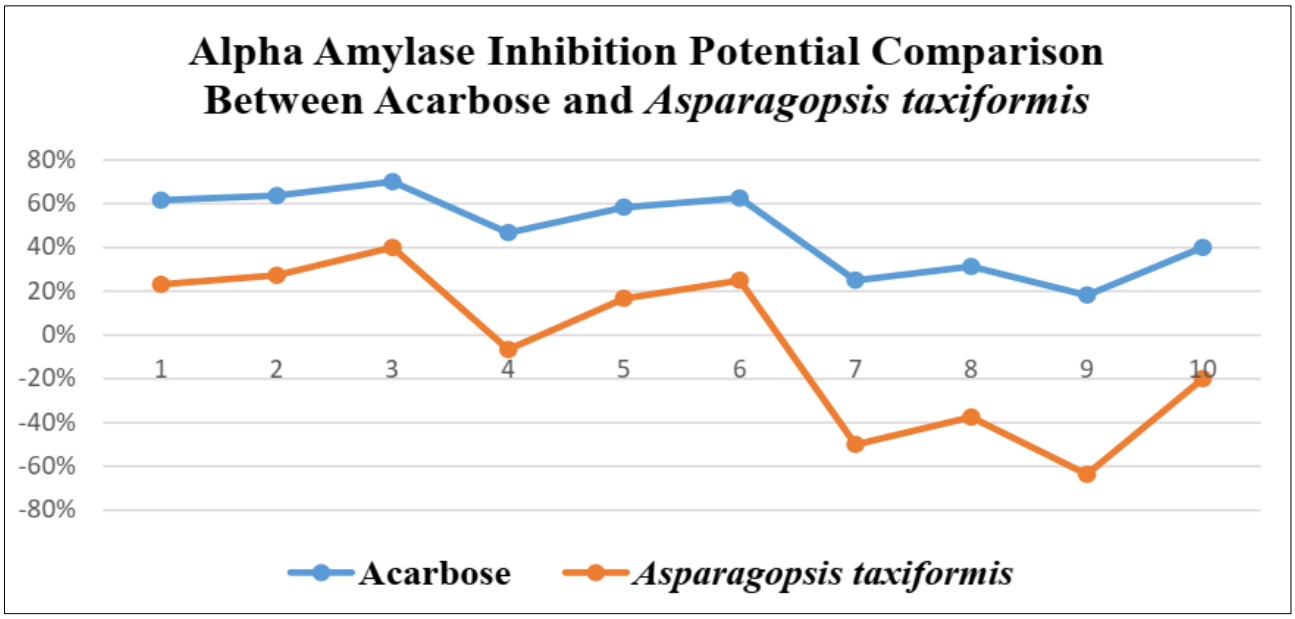

Figure 2: Alpha-amylase inhibitory effect of Acarbose and *Asparagopsis taxiformis*

Effect of 50% Ethanolic Extract of Asparagopsis taxiformis on Diabetic Mice after Acute Treatment

The primary objective of this study was to assess the antihyperglycemic effects of a 50% ethanolic extract derived from *Asparagopsis taxiformis* in mice with alloxan-induced diabetes. The study investigated the impact of various substances on blood glucose levels (BGL) in mice with alloxan-induced diabetes. The substances tested included a 50% ethanolic extract of *Asparagopsis taxiformis*, 50% ethanol, water, and glibenclamide. The effects were measured at different time intervals (0h, 2h, 4h) and different doses. The results, presented in Table 4, demonstrated a decrease in BGL that was both time-dependent and dose-dependent. Statistically significant differences ($p<0.05$) were discovered when comparing the results to those of Group I, which functioned as the Normal Control group. The observed outcome demonstrated minimal impact in the context of immediate intervention.

Table 5 displays the % reduction of blood glucose levels (BGL) in various time durations. The treatment groups that received 50 mg/kg of ethanolic extracts of *Asparagopsis taxiformis* exhibited a greater decrease in plasma glucose levels. In the instance of a 50% ethanol solution, upon administration, the blood glucose level (BGL) exhibited a significant increase, as evidenced by the reduction percentage observed between the 0-hour and 2-hour time points. Subsequently, a progressive reduction in the impact of ethanol was observed. The decrease capability of glibenclamide began to manifest after 2 hours, with regards to positive control. During the acute treatment, no substantial decrease in blood glucose levels (BGL) was seen.

Table 4: Effect of 50% Ethanolic Extract of *Asparagopsis taxiformis* on Diabetic Mice after Acute Treatment

Group	Treatment	BGL (mg/dl) (Mean±SEM)		
		0h	2h	4h
	UT	133.92±9.12	137.52±6.38	115.92±3.76
	DMTH (10 ml/kg)	558.36±16.26[a]	548.64±15.61[a]	513.36±32.92[a]
	DMTE (100 mg/kg)	559.8±34.2[a]	554.04±39.96[a]	544.68±35.42[a]
	DMTE (50 mg/kg)	515.16±43.35[a]	537.84±28.8[a]	461.52±49.69[a]
	DMTG (0.66 mg/kg)	421.56±56.11[a]	441.72±64.68[a]	426.6±69.93[a]
	DMTEt 50% (1ml/kg)	456.48±60.20[a]	522.72±30.14[a]	441.72±57.44[a]

Notes: UT: Untreated; DMTH (10 ml/kg): diabetic control (receiving distilled water 10 ml/kg); DMTE (100 mg/kg): diabetic mice (receiving 100 mg/kg 50% ethanolic extract); DMTE (50 mg/kg): diabetic mice (receiving 50 mg/kg 50% ethanolic extract); DMTG (0.66 mg/kg): diabetic mice (receiving 0.66 mg/kg glibenclamide with dH_2O); DMTEt 50% (1ml/kg): diabetic mice (receiving 1 ml/kg 50% ethanol). [a]Significant values at $p<0.05$ compared to the Group-I.

Table 5: Acute Test Diabetes Reduction Percentage on a Different Time Duration Manner

Group	Treatment	Percent of Reduction		
		0-2 h	2-4 h	0-4 h
	UT	-2.69%	15.71%	13.44%
	DMTH (10 ml/kg)	1.74%	6.43%	8.06%
	DMTE (100 mg/kg)	1.03%	1.69%	2.70%
	DMTE (50 mg/kg)	-4.40%	14.19%	10.41%
	DMTG (0.66 mg/kg)	-4.78%	3.42%	-1.2%
	DMTEt 50% (1ml/kg)	-14.51%	15.5%	3.23%

Effect of Extract of Asparagopsis taxiformis on BGL in Diabetic Mice after Prolonged Treatment

A notable elevation in blood glucose levels (BGL) was observed in chemically induced diabetes mice (using alloxan) compared to the control group of normal mice. Based on the two-way ANOVA analysis results, a significant difference was seen between the diabetic control group and the group that got the conventional medicine, as indicated in Table 6. The results of the post hoc test revealed that glibenclamide exhibited statistically significant reductions in blood glucose levels (BGL) as compared to the diabetic control group ($P<0.05$) on the 7th and 14th days.

Furthermore, administering a 50% ethanolic extract of *Asparagopsis taxiformis* at doses of 100 and 50 mg/kg resulted in a decrease of 9.07% and 12.36% in plasma glucose levels, respectively, after the seventh day of therapy. However, it should be noted that blood glucose levels began to rise again. However, a progressive decline in blood glucose levels (BGL) was noted for the drug glibenclamide, with a reduction of 5.6% recorded during the initial seven-day period, followed by a further fall of 6.66% over days 7 to 14. The blood glucose level (BGL) exhibited a decrease of 11.95% over 14 days. Nevertheless, the ethanol concentration at 50% consistently resulted in a gradual increase in blood glucose levels throughout the study (Table 7). Nevertheless, the administration of glibenclamide and *Asparagopsis taxiformis* did not successfully restore blood glucose levels to the extent observed in the nondiabetic control mice within 14 days.

Table 6: Effect of 50% Ethanolic Extract of *Asparagopsis taxiformis* on the Blood Glucose Level in Alloxan Induced Diabetic Mice after Prolonged Treatment Treatment

Group	Treatment (mg/kg)	BGL (mg/dl)		
		Day 1	Day 7	Day 14
	UT	129.12±5.09	104.28±3.6	122.28±5.33
	DMTH (10 ml/kg)	540.12±15.62[a]	544.92±21.05[a]	518.16±9.16[a]
	DMTE (100 mg/kg)	552.84±36[a]	502.68±28.79[a]	501±15.51[a]
	DMTE (50 mg/kg)	504.84±36.37[a]	442.44±29.57[a]	477.96±13.1[a]
	DMTG (0.66 mg/kg)	429.96±59.12[a]	405.6±63.16[ab]	378.6±38.35[ab]
	DMTEt 50% (1ml/kg)	517.44±10.55[a]	522±10.28[a]	580.56±8.23[a]

Note: [a]Significant values at p<0.05 compared to the Group-I. [b]Significant values at p<0.05 compared to the Group-II

Table 7: Chronic Test Diabetes Reduction percentage

Group	Treatment (mg/kg)	Reduction Percentage		
		Day 1 to 7	Day 7 to 14	Day 1 to 14
	UT	19.24%	-17.26%	5.3%
	DMTH (10 ml/kg)	-0.89%	4.91%	4.07%
	DMTE (100 mg/kg)	9.07%	0.33%	9.38%
	DMTE (50 mg/kg)	12.36%	-8.03%	5.32%
	DMTG (0.66 mg/kg)	5.67%	6.66%	11.95%
	DMTEt 50% (1ml/kg)	-0.88%	-11.22%	-12.2%

Effect of Asparagopsis taxiformis on Body Weight in Chemically Induced Diabetic Mice

After the experimental study, it was observed that the body weights of mice in the normal control group (nondiabetic) exhibited an increase compared to their initial body weights. A notable decrease in body weight was noted in all mice with induced diabetes following the administration of alloxan on the fourth day. The experimental groups, including the positive control group treated with glibenclamide and the groups treated with two different dosages (100 and 50 mg/kg body weight) of extract, shown an increase in body weight compared to the diabetic mice in the control group. However, the body weight of these groups remained lower than that of the normal control group (Figure 3). The mice in Group VI, which were diabetic mice treated with 50% ethanol, could not recover their starting weight after 14 days of therapy. However, the mice in Group II, diabetic mice treated with distilled water, exhibited a steady increase in body weight.

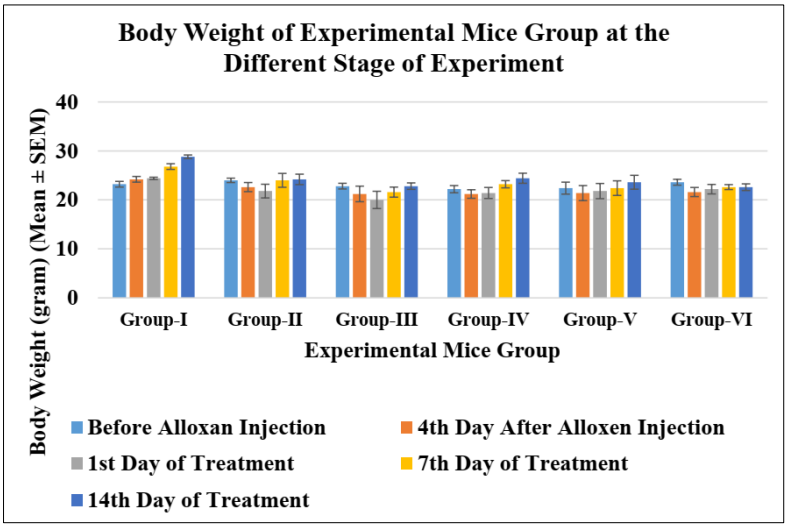

Figure 3: Body Weight of Experimental Mice Group at the Different Stage of Treatment

Determination of the Effect of 50% Ethanolic Extract of Asparagopsis taxiformis on Lipid Profile of Alloxan Induced Diabetic Mice

The impact of a 50% ethanol extract of *Asparagopsis taxiformis* on lipid profile measures in mice with alloxan-induced diabetes as shown in Figure 4. Except low-density lipoprotein (LDL), all lipid profile indicators, including triglycerides (TG), total cholesterol (TC), high-density lipoprotein (HDL), and very low-density lipoprotein (VLDL), exhibited elevated levels in diabetic mice when compared to the normal control group. A statistically significant elevation (p<0.05) in triglyceride (TG) levels was detected in all groups, except group V, which received glibenclamide

treatment. The lipid parameters (triglycerides, cholesterol, high-density lipoprotein, very low-density lipoprotein) in diabetic mice were dramatically elevated when administered 100 and 50 mg/kg body weight of both 50% ethanolic extracts. In contrast, the amount of low-density lipoprotein was significantly reduced compared to normal control group. The administration of ethanolic extracts resulted in a significant increase in triglyceride (TG) levels by 51.90% and 44.36%, cholesterol levels by 9.73% and 6.93%, high-density lipoprotein (HDL) levels by 9.73% and 6.93%, and very low-density lipoprotein (VLDL) levels by 51.90% and 44.36%, respectively, at the doses of 100 and 50 mg/kg body weight. In contrast, a significant reduction in LDL levels was observed in the experimental groups receiving doses of 100 mg/kg and 50 mg/kg of body weight, with reductions of 84.88% and 63.72%, respectively, compared to the control group. A comparable outcome was obtained in the untreated group (Group II) in both concentrations. The administration of the standard medicine glibenclamide at a dosage of 0.66 mg/kg body weight to mice with diabetes resulted in a comparable activity level to that observed in the control group of non-diabetic mice. In contrast to the normal control group, the group of diabetic mice treated with 50% ethanol exhibited a more unfavorable lipid profile.

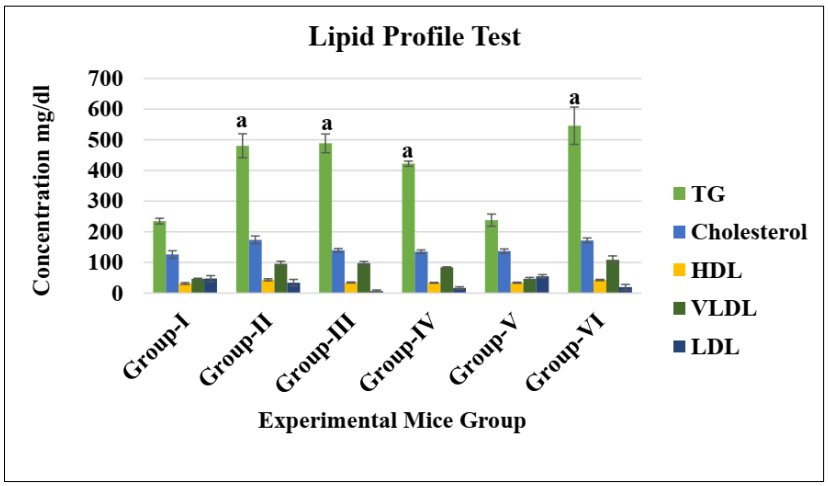

Figure 4: The impact of *Asparagopsis taxiformis* extract on the lipid profile of mice with alloxan-induced diabetes. The values represent the mean ± standard deviation for 5 mice in each group. The bar graph indicating a statistically significant difference at p<0.05

Determination of the Effect of Asparagopsis taxiformis on Serum Creatinine Levels to Access the kidney Function of Alloxan-Induced Diabetic Mice

In diabetic mice the plasma creatinine levels are often found in elevated amount. Creatinine levels serve as significant indications of renal damage in individuals with diabetes (Joy Lindsey *et al.*, 2021). In the present investigation, a 50% ethanolic extract derived from *Asparagopsis taxiformis* was employed to treat alloxan-induced diabetic mice. The outcomes of this study did not reveal any statistically significant variances in creatinine levels, as depicted in Figure 5. The untreated diabetic mice had a notable elevation of 24.93% in creatinine levels, but the other groups did not display a statistically significant rise compared to the normal control group.

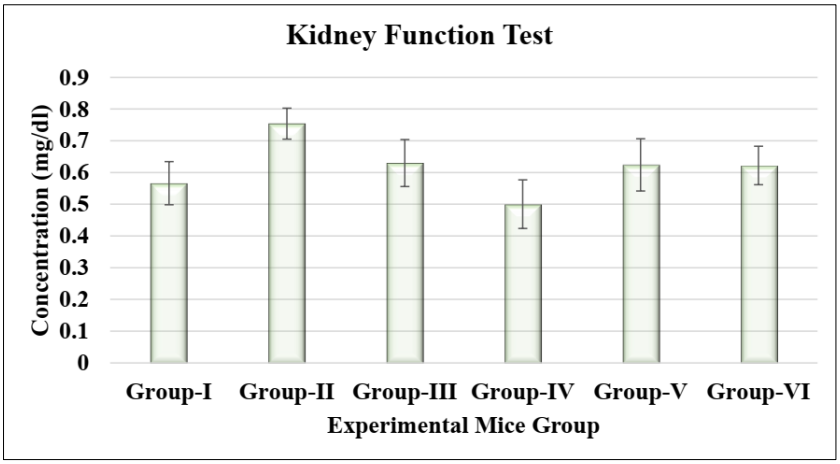

Figure 5: Effect of a 50% ethanolic extract of *Asparagopsis taxiformis* on the serum creatinine level of diabetic mice induced by Alloxan. Mean ± S.D. values are used for presentation.

Determination of the effect of *Asparagopsis taxiformis* on Liver Enzyme Levels of Alloxan-Induced Diabetic Mice

The liver is a major organ affected by diabetes mellitus, hence it is crucial to assess the enzyme levels in the mice that received treatment. The primary enzymes used to assess liver function are serum glutamate-pyruvate transaminase (SGPT) and serum glutamate-oxaloacetate transaminase (SGOT). An increase in the concentration of these enzymes indicates cellular leakage resulting from impaired cell membrane function, thereby contributing to hepatic injury in individuals with diabetes (Joy Lindsey *et al.*, 2021). In this study, it was shown that both the treatment group and untreated mice group exhibited elevated levels of SGPT and SGOT enzymes, except group-VI, when compared to the normal mice group. The SGOT level of group-VI exhibited a substantial increase, suggesting that ethanol can enhance SGOT enzyme levels (Figure 6).

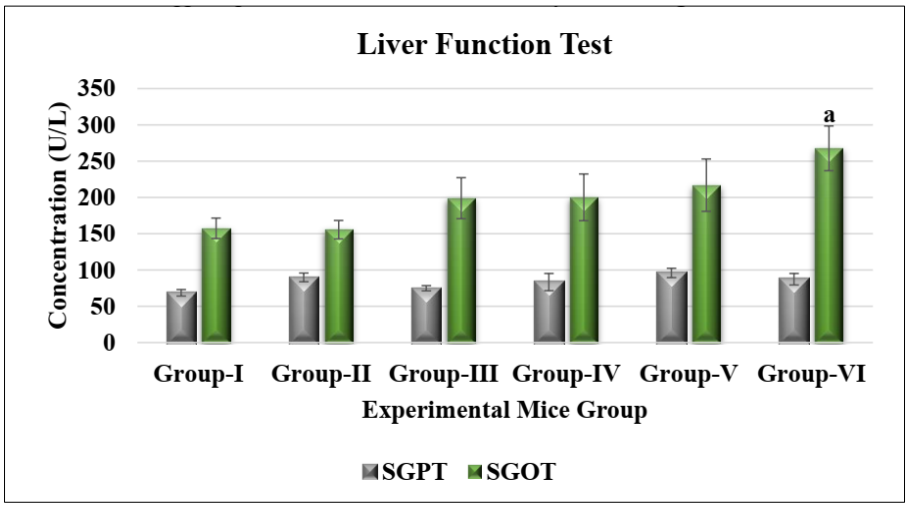

Figure 6: Impact of 50% ethanolic extract of *Asparagopsis taxiformis* on the SGOT and SGPT levels in mice with alloxan-induced diabetes. The values represent the mean ± standard deviation for five mice in each group. Notably different at $p<0.05$

DISCUSSION

Several studies have demonstrated the potential of seaweed in combating diabetes mellitus. Numerous studies have demonstrated the anti-diabetic properties of various secondary metabolites, including coumarins, flavonoids, terpenoids, arginine, and glutamic acid. Extensive research has been conducted on the anti-diabetic properties of brown and red seaweeds, as opposed to green seaweed. The bioactive compounds in marine seaweeds have demonstrated the potential to mitigate diabetes by influencing many pathways, namely by reducing postprandial hyperglycemia and its associated problems (Gunathilaka, Rangee Keertihirathna and Peiris, 2022). To investigate the scientific validity of utilizing *Asparagopsis taxiformis*, a red algae found in the Bay of Bengal, for the treatment of diabetes mellitus, an experimental study was conducted. This study aimed to examine the antihyperglycemic effects of the 50% ethanolic extract, which has not been previously explored, to assess its potential as an anti-diabetic agent. Qualitative and quantitative phytochemical profiling was also performed to further characterize the extract.

The assessment of phytochemicals by chemical assays is necessary to determine the initial phytoconstituents present in extracts. Phytoconstituents have a crucial role in determining the therapeutic effectiveness of a substance. Phytoconstituents, such as alkaloids, flavonoids, glycosides, phenols, and others, exert significant influence in diabetes management (Das *et al.*, 2020). Chemical analyses were conducted on a 50% ethanolic extract of *Asparagopsis taxiformis*, leading to the identification of many bioactive compounds including flavonoids, steroids, phenols, saponins, carbohydrates, amino acids, glycosides, terpenoids, coumarins, and quinones. Additionally, the research employed the UV-visible spectroscopic technique to quantitatively determine the Total Phenolic Content (TPC) and Total Flavonoid Content (TFC). This was done because phenolics and flavonoids, by their structures containing hydroxyl groups, certain double bonds, and ketonic functional groups, primarily function as antioxidants and significantly impact anti-diabetic activity (O *et al.*, 2018). Table 3 provides the quantities of phenolics and flavonoids in the 50% ethanolic extract of *Asparagopsis taxiformis*. The experiment involved the dissolution of a dry sample of 100mg in 1ml of 50% ethanol. The resulting solution contained 0.03mg GAE/ml of total phenolic content (TPC) and 0.006mg CE/ml of total flavonoid content (TFC). These measurements were essential in advancing the progress of the experiment.

The α-amylase inhibitors, sometimes referred to as starch blockers, function by impeding or retarding the assimilation of starch into the human body. This is primarily achieved by inhibiting the hydrolysis process

of 1,4-glycosidic bonds present in starch and other oligosaccharides, preventing the formation of maltose, maltriose, and other forms of simple sugars (Dineshkumar, Mitra and Manjunatha, 2010). This study aimed to assess the inhibitory effect of a 50% ethanolic extract derived from *Asparagopsis taxiformis* on the activities of porcine pancreatic amylase *in-vitro*, in comparison to Acarbose, a commonly prescribed anti-diabetic medication available in the market. Acarbose, when present in the intestinal lumen, exhibits the ability to impede the process of carbohydrate digestion and absorption. This characteristic renders it highly beneficial in treating non-insulin-dependent diabetes mellitus (Islam *et al.*, 2019). The alpha-amylase inhibition capacity of the 50% ethanolic extract of *Asparagopsis taxiformis* was found to be negligible or non-existent compared to acarbose.

Several targets are involved in treating diabetes mellitus, in addition to alpha-amylase. These include the enzymes Dipeptidyl peptidase-4 (DPP4), which breaks down incretin hormones like GLP-1 (glucagon-like peptide-1), which stimulates insulin release and inhibits glucagon secretion; the receptor for Free Fatty Acid Receptor 1 (FFAR1), which regulates glucose and lipid metabolism; the receptor for Peroxisome Proliferator-Activated Receptor gamma (PPARγ), which regulates glucose and lipid metabolism; and α-glucosidase, which aids in the digestion and absorption of carbohydrates. An additional *in-vivo* experiment was conducted to screen for any potential andidiabetic effect of the 50% ethanolic extract of *Asparagopsis taxiformis*.

In this study, mice with alloxan-induced diabetes were given a 50% ethanolic extract of *Asparagopsis taxiformis* at a dose of 100 and 50 mg/kg body weight daily for two weeks. Alloxan, a hydrophilic chemical compound derived from pyrimidine that shares structural similarities with glucose, can produce toxic free radicals through redox cycling reactions that can harm pancreatic β-cells and partially destroy them. This can lead to elevated blood glucose levels or hyperglycemia caused by the pancreas producing insufficient insulin (Islam *et al.*, 2019). No activity was shown in the study's acute therapy, and there was brief but ineffective activity seen in the chronic treatment at a level of 50 mg/kg of body weight. when the diabetes status was gradually alleviated by positive control (glibenclamide treatment). 50% ethanol was used as the negative control since the sample was dissolved in it, and it was discovered that chronic alcohol usage may pose a serious risk to diabetes conditions.

Diabetes causes weight loss because of a lack of insulin, which lowers the amount of protein synthesis because tissues absorb less amino acids, which causes lipolysis in adipose tissues and protein breakdown (Akter, Rahman and Mostofa, 2014). The study's diabetic control mice showed a decrease in weight. However, the diabetic mice's ability to lose weight improved after receiving treatments with *Asparagopsis taxiformis* extract. Extracts from *Asparagopsis taxiformis* may be able to prevent weight loss because of their bioactive components, which stifle the free radicals produced by hyperglycemia. In diabetic mice, it also prevents muscle loss due to inadequate glycemic management and raises body weight (Engeda *et al.*, 2015).

Because of the altered metabolism of lipids and carbohydrates in diabetes mellitus patients, lipid profile imbalance is particularly common. These aberrations are linked to the development of cardiovascular problems in persons with diabetes mellitus as the disease progresses (Laakso *et al.*, 1986; Renard *et al.*, 2004; Parhofer, 2015). Compared to diabetic control mice, the 50% ethanolic extract of *Asparagopsis taxiformis* treated diabetic mice showed decreased LDL levels and increased TG, HDL, and VLDL levels in their serum. These changes did not return to normal. Significant variations were noted in TG. These findings suggest that *Asparagopsis taxiformis* extract does not appear to have any potential benefits for preventing or lessening lipid metabolism-related problems linked to diabetes mellitus. According to the kidney function test, the extract may bring the creatinine down to a normal level.

Patients with persistent diabetes also frequently experience liver damage. By altering the metabolism of lipids, carbs, and proteins, chronic diabetes can cause non-alcoholic fatty liver disease. This condition can then advance to non-alcoholic steatohepatitis, liver cirrhosis, and ultimately hepatocellular carcinomas, which stimulate the inflammatory response to oxidative stress. Patients with DM have elevated levels or activity of liver function enzymes, such as SGPT and SGOT, as a result of liver damage (Renard *et al.*, 2004). Compared to normal control mice, the levels of SGPT and SGOT in diabetic mice were not significantly restored by a 14-day therapy with a 50% ethanolic extract of *Asparagopsis taxiformis*. As a result, this study also shows that *Asparagopsis taxiformis* leaf cannot lower the risk of liver damage and cardiovascular damage linked with diabetes mellitus. The group given 50% ethanol was shown to have significant liver damage in terms of SGOT enzyme level, and the positive control group (glibenclamide) likewise failed to recover the live damage during this treatment time.

Based on a comprehensive analysis of multiple research and the aforementioned experimental results, it can be concluded that the 50% ethanolic extract of *Asparagopsis taxiformis* does not exhibit any anti-diabetic properties. In addition, it demonstrated limited efficacy in restoring liver function and correcting lipid profile imbalances. In addition to posing significant risks to those with diabetes mellitus, alcohol intake has been found to inflict substantial harm onto the liver.

CONCLUSION

In conclusion, the current research focused on the phytochemical profiling and investigation of the anti-diabetic properties of *Asparagopsis taxiformis*, collected from the Bay of Bengal, Bangladesh. The study identified numerous phytochemical constituents in the seaweed through qualitative and quantitative analyses. In contrast to the control group, the results of the *in-vitro* and *in-vivo* tests apparently did not, suggest any anti-diabetic potential. The analysis of biochemical indicators also revealed negative effects, including a harmful impact on the lipid profile and impaired liver function that cannot return to normal. The findings of this study raise concerns about potential risks associated with using *Asparagopsis taxiformis* for its anti-diabetic effects in 50% ethanol, suggesting that additional research in various solvents is necessary to assess these benefits. This emphasizes the necessity of further research to fully comprehend its physiological effects and safety features.

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