

Original Article

## ALPHA AMYLASE AND ALPHA GLUCOSIDASE INHIBITION ACTIVITY OF SELECTED EDIBLE SEAWEEDS FROM SOUTH COAST AREA OF INDIA

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### ABSTRACT

**Objective:** The present work was to investigate the alpha amylase and alpha-glucosidase inhibitory activity of the selected edible seaweeds.

**Methods:** The seaweeds namely *Acanthophora spicifera*, *Gracilaria corticata*, *Gracilaria edulis*, *Ulva lactuca* and *Ulva reticulata* were selected for this study. Six and eight hours of ethanol and aqueous extract were used for the estimation of alpha amylase using DNS method and alpha-glucosidase inhibition activity.

**Results:** The study reported that the solvent from ethanol and aqueous in eight hours of extraction showed a higher inhibitory activity than six hours of extraction. Maximum of 89.1±0.96 and 79.55±3.08 percent of alpha-amylase and alpha-glucosidase inhibition activity were detected in the eight hours of aqueous extract (0.5 ml) of *Ulva reticulata* and *Gracilaria edulis* respectively. All the selected edible seaweeds had significant differences ( $p < 0.05$ ) in alpha amylase and alpha glucosidase inhibition activity between the selected seaweeds with different extracts.

**Conclusion:** It was concluded that all the selected edible seaweeds have the potential to act as a potent inhibitor of the carbohydrate hydrolyzing enzyme. Thus, it was clear from the study that seaweeds incorporated in small amounts in the dishes consumed in the daily diet can bring a control on postprandial blood glucose level.

**Keywords:** Seaweeds, *Ulva reticulata*, Aqueous, Alpha-amylase, Carbohydrate, Anti-diabetic activity, Blood glucose level

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### INTRODUCTION

Diabetes mellitus is a chronic disease, which occurs when the pancreas does not produce enough insulin or when the body cannot efficiently utilize the insulin, it produces [1]. This leads to an increased concentration of glucose in the blood. Diabetes is a significant public health threat and responsible for various long-term complications such as retinopathy, nephropathy and neuropathy leading to severe socio-economic problems throughout the world [2].

According to International Diabetes Federation [3] the prevalence of diabetes among adults (aged 40–59 y) in the world was 382 million in 2013 and this will increase up to 592 million in 2035. Recent national ICMR-INDIAB study reported that more than 62.4 million people are affected with diabetes in India and around 77.2 million people have pre-diabetes. In Tamilnadu, around 4.8 million people are affected with diabetes and 3.9 million people have pre-diabetes [4].

Diabetes requires changes for a lifetime. An important goal of treatment is to provide the patient with the necessary tools to achieve the best possible control of glycemia, lipidemia and blood pressure to prevent, delay or arrest the microvascular and macrovascular complications of diabetes while minimizing hypoglycemia and excess weight gain [5, 6].

The approach for controlling postprandial hyperglycemia is to inhibit the carbohydrate hydrolyzing enzymes such as alpha amylase and alpha glucosidase in the digestive system [7]. Alpha-amylase is involved in the breakdown of long chain carbohydrate and alpha glucosidase breaks down starch and disaccharides to glucose [8]. Inhibition of these enzymes helps to reduce the rate of carbohydrate digestion and cause a reduction in the rate of absorption thereby lowering the postprandial serum glucose levels [9, 10].

The majority of the people in developing countries depend on alternative therapies including natural resources for their primary health care [11]. Zeng Sun and Feng Chen [2] reported that anti-diabetic agents from natural sources have attracted more and more

attention because the clinical trials of several synthetic anti-diabetic drugs have been terminated due to safety concerns. In recent years many marine resources have attracted attention in the search for bioactive compounds to develop new drugs and health foods [12, 13]. Seaweeds are primitive non-flowering plants without root, stem and leaves. They comprise one of the commercially important marine renewable resources [14-16]. Many types of seaweeds have been traditionally used as food in Japan and New Zealand [17]. Seaweeds are a good source of dietary fibres, polysaccharide, polyphenols, polyunsaturated fatty acids, minerals and vitamins. Which possess various biological activities like anti-diabetic, anti-inflammatory, antioxidant activity, antibacterial, anticoagulant antiviral and apoptotic activity [18, 19]. Seaweeds contain a prolific source of bioactive compounds that can be exploited for the treatment of major chronic diseases like diabetes through the inhibition of starch digesting enzymes and the regulation of glucose-induced oxidative stress [20].

Recently, several studies reported that seaweeds can be potential interest for the development of novel drugs, functional foods and pharmaceutical applications. Hence, keeping the above facts in mind, present study was aimed to evaluate the alpha amylase and alpha glucosidase inhibitory activity of selected five edible seaweeds such as *Acanthophora spicifera* (Family-Rhodomelace), *Gracilaria corticata* (Family-Gracilariaceae), *Gracilaria edulis* (Family-Garcilariaceae) from red seaweeds, *Ulva lactuca* (Family-Ulvaceae) and *Ulva reticulata* (Family-Ulvaceae) from green seaweeds.

### MATERIALS AND METHODS

#### Sample collection

Five seaweeds namely *Acanthophora spicifera*, *Gracilaria corticata*, *Gracilaria edulis*, *Ulva lactuca* and *Ulva reticulata* were collected from the south coast area Rameswaram, Tamilnadu, India. The morphological characteristics namely type, colour, texture, shape and habitat of the collected seaweeds were observed and the taxonomical classification of the selected seaweed species was carried out

according to Fritsch [21] and also the collected edible seaweeds were identified by Scientist from Central Salt and Marine Chemicals Research Institute, Mandapam camp, Ramanathapuram, Tamil Nadu, India. Gulf of Mannar, a marine province situated between India and Srilanka. It runs on its Indian side at its head from Pamban islands, a southern coastline that includes the famous pilgrim center Rameswaram to Kanyakumari [22-24]. The collected seaweeds were individually washed and dried. The dried samples were powdered and stored in the high-density polyethylene cover (HDPE).

### Sample preparation

The powdered samples were used for the preparation of the extract with the solvents like ethanol and aqueous (1:10) at 37 °C temperature for six and eight hours at the water bath incubator shaker. The final extract was obtained filtered by what man No.1 filter paper and the filtrate was stored in refrigerator condition, which was used for estimation of alpha-amylase and alpha glucosidase inhibition activity.

### Inhibition of alpha-amylase activity

The inhibition assay was performed according to Miller [25] using DNS method. Aqueous and ethanol extract of seaweed concentration in 0.5 ml were added to 0.5 ml of 0.02 M sodium phosphate buffer (pH6.9 containing 6 mmol sodium chloride) containing 0.04 units of the  $\alpha$ -amylase solution and were incubated at 37 °C for 10 min. After inhibition, 0.5 ml of 1% starch solution in 0.02 M sodium phosphate buffer (pH6.9) was added to each tube. The reaction was stopped with 1.0 ml of 3, 5 DNSA reagent. The test tubes were incubated in a boiling water bath for 5 min, then cooled down to room temperature. The reaction mixture was diluted by the addition of 10 ml distilled water and absorbance was then measured at 540 nm. The control samples were also prepared accordingly without any seaweed extracts and were compared with the test samples containing various concentrations of the seaweed extracts prepared with different solvents. The results were expressed as a percentage (%) inhibition calculated using the formula:

$$\text{Inhibition (\%)} = \frac{\text{Abs (C)} - \text{Abs (Ex)}}{\text{Abs (C)}} \times 100$$

Where Abs (C) is the absorbance of the control (without extract or standard) and Abs (Ex) is the absorbance in the seaweed extracts or standard.

### Inhibition of alpha-glucosidase activity

The inhibitory activity was determined by incubating a solution of starch substrate (2 % w/v maltose or sucrose) 1 ml with 0.2 M Tris buffer pH 8.0 and seaweed extract for 5 min at 37 °C. The reaction was initiated by adding 1 ml of alpha-glucosidase enzyme (1U/ml)

to it followed by incubation for 40 min at 35 °C. Then the reaction was terminated by the addition of 2 ml of 6N HCl. Then the intensity of the colour was measured at 540 nm [26].

$$\text{Inhibition (\%)} = \frac{\text{Abs (C)} - \text{Abs (Ex)}}{\text{Abs (C)}} \times 100$$

Where Abs (C) is the absorbance of the control (without extract or standard) and Abs (Ex) is the absorbance in the seaweed extracts or standard.

### Statistical analysis

The alpha-amylase and alpha glucosidase inhibitory activity were performed in triplicate and mean and standard deviation values were calculated. One way ANOVA followed by a Duncans multiple range test at  $p < 0.05$  using SPSS version 16 to detect the significant differences among the selected seaweeds different extracts.

## RESULTS

### Inhibition of alpha-amylase activity of selected seaweeds

The result of alpha-amylase inhibition activity is presented in table 1. Maximum inhibition 89.1±0.96 percent of alpha amylase was detected in eight hours of aqueous extracts of *Ulva reticulata*. According to the statistical results, the alpha-amylase inhibitory activity of the *Ulva reticulata* in eight hours of the aqueous extract was significantly varied among the selected seaweeds with different extracts except for the eight hours of aqueous extracts of *Gracilaria corticata*, *Gracilaria edulis*, *Ulva lactuca* and the six hours aqueous extracts of the *Gracilaria edulis*. While in the six hours of aqueous extract of *Ulva reticulata* showed 81.96±1.00 percent. The inhibitory activity of *Ulva lactuca* found to be 50.9±0.36 and 59.66±3.91 in six and eight hours of ethanol extracts respectively. Aqueous extract of *Ulva lactuca* showed 80.53±5.00 and 83.43±2.5 in six and eight hours respectively.

Red seaweeds such as *Acanthophora spicifera*, *Gracilaria corticata* and *Gracilaria edulis* showed 27.96±1.05, 62.96±1.69 and 60.26±0.9 percent in six hours and 38.8±6.6, 66.36±4.29 and 75.06±4.20 in eight hours of ethanol extracts respectively. Whereas in aqueous extract it was found to be 49.5±1.73, 80.43±2.5 and 85.2±1.83 percent in *Acanthophora spicifera*, *Gracilaria corticata* and *Gracilaria edulis* extracts taken from six hours and 54.73±5.10, 84.66±1.59 and 87.86±3.4 percent of inhibition detected in the extracts taken eight hours of extraction respectively. *Acanthophora spicifera* had less inhibition of alpha-amylase activity than the *Gracilaria edulis* and *Gracilaria corticata*. The statistical results showed the alpha-amylase inhibition was significantly varied ( $p < 0.05$ ) in *Acanthophora spicifera* in six and eight-hour ethanol extract between the selected seaweeds with different extracts.

Table 1: Alpha-amylase inhibitory activity of selected seaweeds

Nam of the seaweed	Ethanol extract		Aqueous extract	
	Six h extraction	Eight h extraction	Six h extraction	Eight h extraction
<i>Acanthophora spicifera</i>	27.96±1.05 <sup>a</sup>	38.8±6.6 <sup>b</sup>	49.5±1.75 <sup>c</sup>	54.73±5.10 <sup>cd</sup>
<i>Gracilaria corticata</i>	62.96±1.69 <sup>ef</sup>	66.36±4.29 <sup>f</sup>	80.43±2.5 <sup>gh</sup>	84.66±1.59 <sup>hij</sup>
<i>Gracilaria edulis</i>	60.26±0.9 <sup>de</sup>	75.06±4.20 <sup>g</sup>	85.2±1.83 <sup>hij</sup>	87.86±3.4 <sup>hjk</sup>
<i>Ulva lactuca</i>	50.9±0.36 <sup>c</sup>	59.66±3.91 <sup>de</sup>	80.53±5.00 <sup>gh</sup>	83.4±2.5 <sup>hij</sup>
<i>Ulva reticulata</i>	51.03±2.32 <sup>c</sup>	53.46±4.96 <sup>c</sup>	81.96±1.00 <sup>hi</sup>	89.1±0.96 <sup>i</sup>

Values represent the mean of three replicates. Values are given as mean±SD. Values with different alphabets are indicated that the significant differences seen ( $p < 0.05$ ) between the selected seaweeds with different extracts.

### Inhibition of alpha glucosidase activity of seaweeds

The alpha glucosidase activity of the selected seaweeds presented in fig. I. *Acanthophora spicifera* showed 32.0±2.5 and 42.7±4.9 percent of inhibition in six hours of extraction with ethanol and aqueous solvents respectively. Whereas in extracts taken from eight hours of ethanol and aqueous extract it showed 37.08±2.5 and 46.86±10.8 percent respectively. The statistical results showed the alpha glucosidase inhibition was significantly varied ( $p < 0.05$ ) in

*Acanthophora spicifera* in eight-hour ethanol extract between the selected seaweeds with different extracts.

*Gracilaria corticata* showed 51.14±8.61 and 64.34±0.76 percent in six hours and 56±2.68 and 73.53±3.4 percent in eight hours of ethanol and aqueous extract respectively. *Gracilaria edulis* exhibited 55.68±12.3 and 71.91±2.35 percent of inhibition in six hours of ethanol and aqueous extract respectively. While in eight hours of ethanol and aqueous extraction, it was found to have 77.5±2.04 and 79.55±3.08 percent

respectively. The statistical results showed the alpha glucosidase inhibition was significantly varied ( $p < 0.05$ ) in *Gracilaria edulis* in eight-hour aqueous extract between the selected seaweeds with different extracts except the eight-hour aqueous extracts of *Ulva reticulata* and six-hour aqueous extracts of *Gracilaria edulis*.

The alpha glucosidase inhibition activity of *Ulva lactuca* was  $50.42 \pm 3.8$  and  $65.71 \pm 1.21$  in six and eight hours of ethanol extract respectively. Whereas in aqueous extract of *Ulva lactuca* from six and eight hours extraction showed  $43.5 \pm 8.5$  and  $61.81 \pm 1.83$  percent of alpha glucosidase inhibition respectively. In *Ulva reticulata* it was found to be  $30.3 \pm 2.1$  and  $43.5 \pm 4.78$  percent in six and eight hours of ethanol extract taken from extraction respectively. Whereas it showed  $67.4 \pm 4.73$  percent inhibition in six hours and  $79.55 \pm 3.08$  percent of inhibition in the extract of eight-hour extraction using

aqueous solvent. The statistical results showed the alpha glucosidase inhibition was significantly varied at  $p < 0.05$  percent between the seaweeds with different extracts.

The alpha-amylase and alpha glucosidase activities of the selected seaweeds were found to be in the range from  $27.96 \pm 1.05$  to  $89.1 \pm 0.96$  and  $32.0 \pm 1.5$  to  $79.55 \pm 3.08$  respectively. The maximum inhibition of alpha-amylase and alpha glucosidase activity were detected in the eight hours of aqueous extracts of *Ulva reticulata* and *Gracilaria edulis* respectively. The minimum inhibition was detected in *Acanthophora spicifera*. The study reported that all the selected edible seaweeds have the capacity to inhibit alpha-amylase and alpha glucosidase activity. The standard Acarbose showed  $58.2 \pm 1.2$  and  $78.4 \pm 0.5$  percent of alpha-amylase and alpha glucosidase inhibition respectively.

**Table 2: Alpha glucosidase inhibitory activity of selected seaweeds**

Nam of the seaweed	Ethanol extract		Aqueous extract	
	Six h extraction	Eight h extraction	Six h extraction	Eight h extraction
<i>Acanthophora spicifera</i>	$32.0 \pm 2.5^a$	$37.08 \pm 2.5^b$	$42.7 \pm 4.9^c$	$46.86 \pm 10^{cd}$
<i>Gracilaria corticata</i>	$51.14 \pm 8.61^d$	$56 \pm 2.68^e$	$64.34 \pm 0.76^g$	$73.53 \pm 3.4^{hi}$
<i>Gracilaria edulis</i>	$55.68 \pm 12.3^e$	$71.91 \pm 2.35^h$	$77.5 \pm 2.04^j$	$79.55 \pm 3.08^j$
<i>Ulva lactuca</i>	$50.42 \pm 3.8^d$	$65.71 \pm 1.21^g$	$43.5 \pm 8.5^c$	$61.81 \pm 1.83^f$
<i>Ulva reticulata</i>	$30.3 \pm 2.1^a$	$43.5 \pm 4.78^c$	$67.4 \pm 4.73^g$	$76.02 \pm 0.83^{hij}$

Values represent the mean of three replicates. Values are given as mean  $\pm$  SD. Values with different alphabets are indicated that the significant differences seen ( $p < 0.05$ ) between the selected seaweeds with different extracts.

## DISCUSSION

The dramatic increase in the incidence of type 2 diabetes represents one of the most significant global health issues of the Twenty-first century has prompted revisions in the prevention and treatment of this devastating disease [27]. Two enzymes that play a key role in diabetes are alpha amylase and alpha glucosidase. Alpha-amylase catalyzes the hydrolysis of starch to a mixture of smaller oligosaccharides consisting of maltose, maltotriose and oligo glucans and it is further degraded to glucose by  $\alpha$ -glucosidase after which they are absorbed into the bloodstream [28]. Inhibition of alpha glucosidase enzyme activity leads to a reduction in disaccharide hydrolysis that has beneficial effects on glycemic index control in diabetic patients [29, 30]. Recent studies have also shown that marine algae contain a wide range of highly bioactive secondary metabolites with therapeutic potential that might represent useful leads in the development of functional ingredients for nutraceuticals, cosmeceuticals, and pharmaceuticals [31-33].

In the present study, the anti-diabetic activity of five edible seaweeds collected from the Gulf of Mannar was evaluated. The south coast of Tamil Nadu in India is a unique marine habitat infested with diverse seaweeds. Seaweeds are the extraordinary, sustainable resources in the marine ecosystem that have been used as a source of food, medicine and industrial products [34].

Three seaweeds from red algae (*Acanthophora spicifera*, *Gracilaria corticata* and *Gracilaria edulis*) and two seaweeds from green algae (*Ulva lactuca* and *Ulva reticulata*) used in this study. Alpha-amylase and alpha glucosidase inhibition activity were identified using the solvents like ethanol and aqueous in six and eight h of extraction.

Xu [35] reported that seaweeds are known to contain alpha glucosidase and alpha amylase inhibitors. The study reported that all the selected seaweed extracts found have a valuable amount of alpha amylase and alpha glucosidase inhibitory capacity. The *in vitro* alpha amylase and alpha glucosidase activity of aqueous extracts of seaweeds showed a higher degree of inhibitory activity than ethanol extract. The study compares the anti-diabetic activity with the duration of solvent extraction of six and eight h and it is reported that the eight h extraction of both solvents namely ethanol and aqueous were found to have an increased inhibition of alpha-amylase and alpha glucosidase activity than the six h of extraction.

Zheng and Feng Chen [2] evaluated the inhibitory effects of green algae *Chlorella pyrenoidasa* possessed moderate alpha-amylase inhibitory activity and strong alpha glucosidase inhibitory activity

and concluded that the green algae considered as a potential functional food for diabetic patients.

In the study conducted the maximum inhibition of alpha-amylase and alpha glucosidase was recorded in the *Ulva reticulata* and *Gracilaria edulis* followed by *Ulva lactuca* and *Gracilaria corticata* in extracts of eight-hour extraction in both solvents. It is clear that at the time of extraction increases the activity as increased this indicates that seaweed required a longer time to release the phytochemicals and colloids from its tissues. The alpha-amylase inhibitory activity of the selected seaweed extracts showed the higher inhibition compare, then the standard Acarbose except for the *Acanthophora spicifera*. However, the alpha glucosidase inhibitory activity of the standard Acarbose was higher than the selected seaweeds extracts except the *Gracilaria edulis* with eight h of aqueous extracts.

*Ulva* species are used as a traditional food in many Asian countries. In Japan, they are included in a variety of dishes such as salads, soups, cookies, meals and condiments as well as a mixed product with other green macroalgae [36, 37]. Soluble fiber levels are high in *Ulva* Species from 20 to 40 percent of dry weight. Recently several studies reported that *Ulva* could be of potential interest for food, development of novel drugs and functional foods, pharmaceutical and agricultural applications [38, 39].

Lakshmana Senthil [40] on screening the alpha-amylase inhibitory activity using different solvents in ten seaweeds from the southeast coastal area of India found that the ethyl extracts of *C. ramcesmosa*, *G. gracilis*, *C. harnemanii* and *P. gymnospora* showed potent alpha-amylase inhibitory activity. Among all the seaweeds *Gracilaria edulis* showed inhibition activity of  $57 \pm 0.9$ ,  $53 \pm 0.1$  and  $60 \pm 1.2$  in extracts taken from different solvents like methanol, acetone and ethyl acetate respectively. This study also determined the effective percent of inhibition detected in ethanol and aqueous extracts of *Gracilaria edulis*.

Hang Wang [41] stated that a diet rich in marine products lowers the incidence of diabetes, cancer and obesity. Lucy [42] reported that the polyphenolic-rich extracts from edible seaweed may have anti-diabetic effects due to their ability to inhibit certain digestive enzymes and were responsible for the stimulation on glucose uptake [43, 44].

In the present study, it is clear that seaweeds may take a longer time to digest and stays for a longer period of time in the gastro intestine and the fiber and phenolic contents may interfere with the absorption which in turn delays rise in blood glucose levels. Min Sun

Kim [44] evaluated the physiological effects of seaweed supplementation in subjects with diabetes mellitus. Subjects were randomised into either a control group or an experimental group. Pills with equal parts of sea tangle and sea mustard (48 gm) were provided to the experimental group three times in a day for four weeks and the study reported that the fasting blood glucose levels and two-hour postprandial blood glucose measurements were decreased significantly in the experimental group. This study concludes that ingestion of seaweed influences glycemic control, lower blood lipids and increased antioxidant enzyme activities.

Henricksen [45] reported that oxidative stress known to play a significant role in the development and progression of diabetes mellitus. Free radicals, including superoxide's and hydroxyl radicals, induce the structural modification and functional impairment of key molecules during the glycation process, leading to the onset of diabetes mellitus [46]. Algal polysaccharides have demonstrated to play an important role as free radical scavengers for the prevention of damage from oxidative stress in living organisms [35, 47-49]. A reduction in the Glycemic Index of starch-based foods can be obtained with the use of fibers, which could be beneficial to diabetic patients by aiding in the control of postprandial insulin release [50] Lahaye and Kaferer [51] stated that the carbohydrate content of algae is relatively high (33.75% DM) but algae are not considered as an energy rich food because the digestibility of these carbohydrates is low.

Suhaila Mohamed [52] reported that most of the seaweeds contain high levels of soluble dietary fibers (carrageenan, agar, alginates). They possess a wide range of beneficial physiological functions, including increased satiety; increased gut transit time reduced cholesterol and glucose absorption in the gastrointestinal tract and reduced risk of coronary heart disease. Above studies are clear that several types of seaweeds are rich in dietary fiber and other secondary metabolites and their mechanism, it may be responsible for the potential anti-diabetic activity of the seaweeds, which inhibit the alpha amylase, and alpha glucosidase activity.

## CONCLUSION

Diabetes mellitus is a global health threat among the degenerative diseases. To regulate the postprandial glucose level is important in the treatment of diabetes mellitus. The inhibition of key enzymes of carbohydrate digestion such as alpha amylase and alpha glucosidase helps to maintain the postprandial blood glucose level. Most of the studies have reported that seaweeds possess an inhibitory activity of the enzymes due to their soluble fiber, phenolic compounds and other pharmaceutical compounds. It may act as a low glycemic index food ingredient and help to maintain a blood glucose level through slow digestion and absorption. From the study, it is concluded that all the selected seaweeds possess an inhibitory activity of alpha amylase and alpha glucosidase. It can be used as a functional food in product development to maintain the postprandial blood glucose, which may prevent or delay the complications related diabetes.

## CONFLICT OF INTERESTS

Declared none

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