ABSTRACT
Aqueous extracts of four seaweeds collected from Gulf of Mannar coastal waters were tested for α-amylase and α-glucosidase inhibition properties. The aqueous extracts of seaweeds in the order of Gracilaria edulis, Sargassum polycystum, Ulva lactuca and Gracilariar corticata showed significant inhibitory activity against α-amylase and α-glucosidase enzymes. G. edulis was found to be a potent inhibitor of α-glucosidase with an IC₅₀ value of 46μg/mL. The aqueous extract of S. polycystum at a concentration of 10-100 μg/ml showed maximum α-amylase inhibitory activity with an IC₅₀ value of 60μg/mL. This study warrants further investigation on the antidiabetic activity and identifies the hyperglycemic principle to elucidate their mode of action.

KEY WORDS: Seaweeds, α-glucosidase, α amylose inhibitory activity.

INTRODUCTION
Diabetes mellitus is a metabolic disorder characterized by increased blood glucose levels with instability in carbohydrate, fat and protein metabolism. One therapeutic approach for treating diabetes is to decrease postprandial hyperglycemia. This can be attained by delaying the absorption of glucose through the inhibition of carbohydrate hydrolyzing enzymes, α-amylase and α-glucosidase in the digestive track. The α-glucosidase inhibitors can retard the liberation of glucose from dietary complex carbohydrates and delay glucose absorption, resulting in reduced postprandial plasma glucose levels and suppress postprandial hyperglycemia. Hyperglycemia defining established diabetes can induce oxidative stress by various mechanisms. Oxidative stress has been shown to have a significant effect in the causation of diabetes as well as diabetes related complications. The α-glucosidase enzymes are responsible for breakdown of carbohydrates to absorbable monosaccharide, α-glucosidase enzymes delay the absorption of ingested carbohydrates, reducing the postprandial glucose and insulin peaks. Plants have always been an excellent source of drugs and many of the currently existing drugs have been derived directly or indirectly from them. Previous study shows that seaweeds are known to contain α-glucosidase inhibitors. The current study was conducted to find out α-glucosidase and α-amylase inhibitory effect of selected seaweeds. Majority of marine algae from Gulf of Mannar, southeast coast of Tamilnadu, India were left unexplored for bioactive substances. There are no previous reports of any in vitro α-glucosidase and α-amylase inhibitory activity of Gracilaria edulis, Sargassum polycystum, Ulva lactuca and Gracilaria corticata. In the present study, we describe the α-amylase and α-glucosidase enzyme inhibitory activity of G. edulis, S. polycystum, U. lactuca and G. corticata aqueous extracts obtained from the Gulf of Mannar southeast coast of Tamilnadu, India.

MATERIALS AND METHODS
All chemicals were purchased from Sigma-Aldrich (USA) unless otherwise stated. The chemicals were of analytical grade.

SAMPLE COLLECTION
In the present study, seaweeds Ulva lactuca Linn (Chlorophyceae), Sargassum polycystum C.Agardh (phaeophyta), Gracilaria edulis (S.G.Gmelin) P.C.Silva (Rhodophyta), and Gracilaria corticata (J.Agardh) J.Agardh (Rhodophyta) was collected from Mandapam coastal region (78°8’E, 9°17’N), in Gulf of Mannar, Tamilnadu, South India on low tide during December 2009 and immediately brought to the laboratory in polythene bags and washed several times with tap water to remove sand, mud and attached fauna. The seaweeds were cleaned using brush for the removal of the epiphytes with distilled water. After cleaning, algae were dried in shade at room temperature for one week. The dried algal samples were homogenized to fine powder and subjected to extraction.

PREPARATION OF EXTRACTS
Five hundred grams of powdered seaweed samples were taken and extracted with water using soxhlet apparatus. The crude extracts were later concentrated under reduced pressure to get their corresponding residues. The yield of the extract was around 7.7%. The aqueous extracts were further subjected for α-glucosidase and α-amylase enzyme inhibitory assay. All the assays were conducted in triplicate.

α-AMYLASE ENZYME INHIBITION ASSAY
The α-amylase activity was determined by the method of Hansawasdi et.al. (2000) ⁹. Starch azure (2mg) was suspended in each of the tubes containing 0.2ml of 0.5 M Tris-HCl buffer (pH 6.9) and 0.01 M CaCl₂. The tubes containing substrate solution were boiled for 5 min and were then incubated at 37°C for 5 min. Seaweed extract (0.2ml) was taken in each tube containing different concentrations (10, 20, 40, 60, 80 and 100 μg/ml) of dimethyl sulfoxide. Porcine pancreatic amylase (PAA) was dissolved in Tris-HCl buffer to form a concentration of 2units/ml and 0.1 ml of this enzyme solution were added to each of the above mentioned tubes. The reaction was carried out at 37°C for 10 min and was stopped by adding 0.5 ml of 50% acetic acid in each tube. The reaction mixture was centrifuged at 3000 rpm for 5 min at 4°C. The absorbance of the resulting supernatant was measured at 595 nm using a spectrophotometer (UV-Vis spectrophotometerUV-2450 (Shimadzu)). The α-amylase inhibitory activity was calculated as follows:
amylase

The activity in controls (with an IC50 value of 67 µg/ml) was second most active of the different seaweed species were compared on the basis of their glucosidase inhibitory potential. The α-glucosidase and α-amylase inhibitory effect of aqueous extract of the four seaweeds: U. lactuca, S. polycystum, G. edulis, and G. corticata were studied to find out the possible mechanism of its anti-diabetic action. Among the four sea weeds studied aqueous extract of G. edulis showed sufficient α-glucosidase and S. polycystum showed better α-amylase enzyme inhibition property. α-glucosidase inhibitors have a potential for the treatment of diabetes because they reduce diet-induced hyperglycemia. The extracts from some macro algae such as Rhodomela confervoides (Huds.) Silva, Gracilaria tectorii (Suringham) De Toni, Plocamium teflariae Harv., Dictyopteris divaricata (Okam.) Okam,Ulval pertusa and Enteromorpha intestinalis (L.) reported for the strong inhibitory activity of alpha-glucosidase. In conclusion, results obtained in the present study supports the use of G. edulis as a dietary supplement for the treatment of diabetes. Further work to investigate the effects of these extracts in diabetic rats may shed light on the hypoglycemic effects of the extracts.

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REFERENCES


**Figure 1:** In vitro α-glucosidase inhibitory activity of seaweeds

**Figure 2:** In vitro α-amylase inhibitory activity of seaweeds

**Table 1.** IC₅₀ value of α-glucosidase and α-amylase aqueous extracts of seaweeds

<table>
<thead>
<tr>
<th>S.No</th>
<th>Seaweeds</th>
<th>IC₅₀ value (µg/ml)</th>
<th>α-glucosidase inhibition activity</th>
<th>α-amylase inhibition activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>G. edulis</td>
<td>46 (µg/ml)</td>
<td></td>
<td>83 (µg/ml)</td>
</tr>
<tr>
<td>2.</td>
<td>S. polycystum</td>
<td>50 (µg/ml)</td>
<td></td>
<td>60 (µg/ml)</td>
</tr>
<tr>
<td>3.</td>
<td>U. lactuca</td>
<td>53 (µg/ml)</td>
<td></td>
<td>67 (µg/ml)</td>
</tr>
<tr>
<td>4.</td>
<td>G. corticata</td>
<td>87 (µg/ml)</td>
<td></td>
<td>82 (µg/ml)</td>
</tr>
</tbody>
</table>

Results represented the mean of independent triplicate experiments.

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