α-AMYLASE AND α-GLUCOSIDASE INHIBITION BY BROWN SEAWEED (Sargassum sp) EXTRACTS

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ABSTRACT
Inhibition of intestinal α-amylase and α-glucosidase is an important strategy to control post-prandial hyperglycemia associated with diabetes mellitus. In vitro inhibitory effects of crude extracts of seaweed against α-amylase and α-glucosidase were studied. Crude ethyl acetate extracts of Sargassum aquifolium were the stronger inhibitor to α-amylase and α-glucosidase than others. Furthermore, Sargassum aquifolium ethyl acetate extract significantly suppressed the rise in postprandial glucose level after oral administration of glucose in normal rats. The results of this study suggest that the crude Sargassum aquifolium extract may suppress the rise in postprandial hyperglycemia in vivo in part, through inhibition of alpha amylase and glucosidase.

Keywords: α-amylase and α-glucosidase, in vitro, rats, hypoglycemia.

INTRODUCTION
Hyperglycemia, a condition characterized by an abnormal post-prandial increase in the blood glucose level, has been linked to the onset of type 2 diabetes and associated with oxidative dysfunction and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels, and has been shown to be also linked to hypertension (Haffner 1998). Hydrolysis of dietary carbohydrates such as starch is the major source of glucose in the blood. This hydrolysis is carried out by a group of hydrolytic enzymes that includes pancreatic α-amylase and intestinal α-glucosidases (Harris and Zimmer 1992; Bischoff 1994). It is believed that inhibition of these enzymes can be an important strategy for management of type 2 diabetes (Krentz and Bailey 2005), wherein α-glucosidase inhibitors could retard the rapid utilization of dietary carbohydrates and suppress postprandial hyperglycemia (Watanabe and others 1997). Currently, therapeutic drugs, such as acarbose, are shown to effectively reduce the intestinal absorption of sugar in human (Jenkins and others 1981; Cheng and Fantus 2005).

The main drawback of acarbose is its side effects such as abdominal distention, flatulence, meteorism, and possibly diarrhea (Bischoff and others 1985). It has been suggested that such adverse effects might be caused by the excessive inhibition of pancreatic α-amylase resulting in the abnormal bacterial fermentation of undigested carbohydrates in the colon (Bischoff and others 1985; Horii and others 1987). Certain plant extracts, such as cranberry, rhodiola, oregano, and rosemary, were found to have lower inhibitory effect against α-amylase activity and stronger α-glucosidase inhibitory effect and therefore can be potentially used as an effective therapy for postprandial hyperglycemia with minimal side effects (Horii and others 1987; Apostolidis and others 2006; Kwon and others 2006).

Recent statistical data show that type 2 diabetes is a global health challenge. Studies indicate that the total prevalence of diabetes in United States alone is 20.8 million (almost 7% of the total population), when in 1980 and 2003 this number was 5.8 and 13.8 million, respectively (Centers for Disease Control and Prevention 2005). Management of this disease will require multi-purpose strategies, including dietary chemoprevention as part of the solution.

The α amylase and glucosidase are responsible for breakdown of carbohydrate to absorbable monosaccharide. These enzymes delay the absorption of ingested carbohydrates, reducing the postprandial
glucose and insulin peaks (Stuart et al. 2004). 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 these enzymes inhibitor (Kim et al. 2008). Zhang et al. (2007) have determined that the solvent extracts of brown algae have high phenolic content and that this phenolic content correlates with reduction of blood glucose levels in rats.

The current study was conducted to find out α amylase and α glucosidase inhibitory effect of Sargassum sp. Majority of Sargassum sp from Talango Island, Sumenep District, East Java, Indonesia were left unexplored for bioactive substances. There are no previous reports of any in vitro α amylase and α glucosidase activity.

MATERIALS AND METHODS

Fresh Sargassum sp were harvested from the Talango shoreline on July 2012. α-Amylase (porcine pancreatic, EC 3.2.1.1), α-glucosidase (yeast, EC 3.2.1.20), and acarbose (yeast, EC 260.030.7) were purchased from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). Acarbose is a known α-glycosidase and α-amylase inhibitor currently used for type 2 diabetes management. Unless otherwise specified, all chemicals were also purchased from Sigma Chemical Co.

Sample preparation.

In the initial screening, S. filipendula, S. aquifolium, S. siliquosum, S. polycystum and S. duplicatum (harvested in July 2012) were dried overnight at 100 °C in oven. The dried seaweeds were then ground for 2 min at high speed using a Stephan UMS (Germany). The resulting dried powder (5 g) was extracted with 20 mL ethyl acetate at 90°C using a reflux condenser for 30 min. The supernatant was collected and centrifuged at 7200 × g using a Fisher Scientific MicroV microcentrifuge (Pittsburgh, Pa., U.S.A.) to determine the total phenolic content.

For the next step, we decided to use a simpler extraction method using fresh S. aquifolium because of its highest phenolic content among seaweeds analyzed. Extracts were prepared from 50 g of fresh S. aquifolium (harvested in August 2012) with 200 mL ethyl acetate at room temperature by blending for 1 min in an 1.25 L Osterizer blender. The blended product was filtered through a nr 25 sieve (710 μm) with 15 kg weight pressure on top for 5 min and the filtrate was centrifuged at 7200 × g and then used to determine the optimum extraction ratio. After extraction, all samples were stored in a −18 °C freezer and all experiments were performed within 3 wk.

Total phenolics assay

The total phenolics were determined following the procedure modified from Shetty and others (1995). Briefly, 1 mL extract was transferred into a test tube and mixed with 1 mL 95% ethanol and 5 mL distilled water. To each sample, 0.5 mL 50% (v/v) Folin–Ciocalteu reagent was added and vortex mixed. After 5 min, 1mL 5% Na2CO3 was added to the reaction mixture and allowed to stand for 60 min. The absorbance was read at 725 nm using a Perkin-Elmer Lambda 4b spectrophotometer (Waltham, Mass., U.S.A.). The absorbance values were converted to total phenolics and were expressed in mg gallic acid/g sample fresh weight (FW). Standard curve was established using various concentrations of gallic acid in ethanol.

α-amylase Inhibition Assay

A mixture of 500 μL extract or acarbose and 500 μL 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) containing α-amylase solution (13U/mL) were incubated at 25 °C for 10 min. After preincubation, 500 μL 1% soluble starch solution in 0.02M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) were added to each tube at timed intervals. The reaction mixtures were then incubated at 25°C for 10 min followed by addition of 1 mL dinitrosalicylic acid color reagent. The test tubes were then placed in a boiling water bath for 5 min to stop the reaction and cooled to room temperature. The reaction mixture was then diluted with
10mL distilled water and absorbance was read at 540 nm.

\[
\frac{\Delta \text{Abs}_{\text{control}} - \Delta \text{Abs}_{\text{sample}}}{\Delta \text{Abs}_{\text{control}}} \times 100
\]

The inhibitory activity was expressed as the half maximal inhibitory concentration (IC50), which is a measure of the effectiveness of a compound in inhibiting biological or biochemical function.

**α-glucosidase inhibition assay**

A mixture of 50 μL extract or acarbose solution and 100 μL of 0.1M phosphate buffer (pH 6.9) containing α-glucosidase solution (1.0 U/mL) was incubated in 96 well plates at 25°C for 10min. After preincubation, 50 μl of 5 mM p-nitrophenyl-α-D-glucopyranoside solution in 0.1M phosphate buffer (pH6.9) were added to each well at timed intervals. The reaction mixtures were incubated at 25°C for 5min. Before and after incubation, absorbance was recorded at 405 nm by micro-plate reader (VMax, Molecular Device Co., Sunnyvale, Calif., U.S.A.) and compared to that of the control which had 50 μL buffer solution in place of the extract. The α-glucosidase inhibitory activity was expressed as inhibition percent and was calculated as follows:

\[
\frac{\Delta \text{Abs}_{\text{control}} - \Delta \text{Abs}_{\text{sample}}}{\Delta \text{Abs}_{\text{control}}} \times 100
\]

The inhibitory results were expressed as the half maximal inhibitory concentration (IC50), which is a measure of the effectiveness of a compound in inhibiting biological or biochemical function.

**Statistical analysis**

All experiments were performed twice and analysis for each experiment was carried out in triplicate. Means, standard deviations, the degree of significance (P<0.05—one way analysis of variance [ANOVA] and t-test) and correlation (r—Pearson correlation coefficient) were determined using software SPSS 16.1. IC50 values were calculated using ED50plus vol.1 developed by Vargas. (http://www.softlookup.com/display.asp?id=2972, accessed May 2009).

**RESULTS AND DISCUSSION**

**Total phenolic content**

The initial screening of the different seaweeds showed that *S. aquifolium* had the highest total phenolic content (6.8 mg/g dry weight) and those of the other 3 ranged from 1.3 to 1.6 mg/g dry weight (Table 1). Therefore, further evaluation was focused on *S. aquifolium*.

**Table 1. Polyphenol content (eq mg gallic acid/g extract) on several extract of Sargassum sp**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Solvents</th>
<th>n-Hexana</th>
<th>Ethyl acetate</th>
<th>Ethanol</th>
<th>Aquadest</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. filipendula</em></td>
<td>14,561</td>
<td>21,154</td>
<td>6,650</td>
<td>2,343</td>
<td></td>
</tr>
<tr>
<td><em>S. aquifolium</em></td>
<td>13,678</td>
<td>46,447</td>
<td>20,227</td>
<td>1,421</td>
<td></td>
</tr>
<tr>
<td><em>S. siliquosum</em></td>
<td>12,505</td>
<td>23,631</td>
<td>20,430</td>
<td>4,048</td>
<td></td>
</tr>
<tr>
<td><em>S. polycystum</em></td>
<td>10,138</td>
<td>43,486</td>
<td>7,828</td>
<td>3,257</td>
<td></td>
</tr>
<tr>
<td><em>S. duplicatum</em></td>
<td>10,690</td>
<td>27,045</td>
<td>8,895</td>
<td>2,151</td>
<td></td>
</tr>
</tbody>
</table>

**α-glucosidase inhibition assay**

α-Glucosidase inhibitory activity was observed in all tested samples (Table 2). The increase of α-glucosidase inhibitory activity correlated well with the total phenolic contents (r=−0.99). Previous reports have shown that phenolic phytochemicals from plant sources could be effective α-glucosidase inhibitors (Apostolidis and others 2006; Kwon and others 2006). Our results suggest that this inhibitory activity is due to the phenolic compounds present in *S. aquifolium* although the specificity of the inhibitory effect was not evaluated in this study. It is important to point out that acarbose is a chemical drug specifically designed for α-glucosidase inhibition and has various side effects (Bischoff and others 1985).

**Table 2. Inhibition (%) of α amylase activity by extract of Sargassum sp**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Solvents</th>
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<th>Ethyl acetate</th>
<th>Ethanol</th>
<th>Aquadest</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. filipendula</em></td>
<td>18</td>
<td>27</td>
<td>5</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>
α-amylase inhibition assay

α-Amylase inhibitory activity was observed in all tested samples (Table 3). Similar to α-glucosidase inhibitory activity, α-amylase inhibitory activity increased with an increase in total phenolic content (r =-0.88) although the specificity of the inhibitory effect was not evaluated in this study. The α-amylase inhibitory activity of S. aquifolium appears to be much lower than α-glucosidase inhibitory activity. Teixeira and others (2007) have reported the potential of acetone extract of brown seaweed (Spatoglossum Schroederi) for α-amylase inhibition (IC_{50} 580 μg/mL). Previous reports have indicated that plant-derived phenolic phytochemicals have lower α-amylase inhibitory activity and a stronger inhibition activity against α-glucosidase (Apostolidis and others 2006; Kwon and others 2006).

The main side effects of type 2 diabetes control drugs, such as acarbose, are abdominal distention, flatulence, meteorism, and possibly diarrhea (Bischoff and others 1985). It has been suggested that such adverse effects might be caused by the excessive inhibition of pancreatic α-amylase resulting in the abnormal bacterial fermentation of undigested carbohydrates in the colon. (Bischoff and others 1985; Horii and others 1987). In our study, although we observed a lower α-amylase inhibitory activity than acarbose, we cannot rule out potential side effects. However, the lower in vitro α-amylase inhibitory activity suggests that the extent of the side effects will be less than acarbose.

**Tabel 3.** Inhibition (%) of α-glucosidase activity by extract of Sargassum sp

<table>
<thead>
<tr>
<th>Sample</th>
<th>n-Hexana</th>
<th>Ethyl acetate</th>
<th>Ethanol</th>
<th>Aquadest</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. filipendula</td>
<td>23</td>
<td>37</td>
<td>10</td>
<td>4.5</td>
</tr>
<tr>
<td>S. aquifolium</td>
<td>20</td>
<td>65</td>
<td>26</td>
<td>3.4</td>
</tr>
</tbody>
</table>

Conclusions

The initial screening showed that the brown seaweed S. aquifolium extract has the highest total phenolic contents among locally harvested seaweeds studied. S. aquifolium ethyl acetate extracts have a good inhibitory potential on carbohydrate metabolizing enzymes, especially α-glucosidase. These inhibitory activities increased with phenolic content and the best inhibitory potential was observed with the highest amount of phenolic phytochemicals. This study provides strong *in vitro* evidence for potential α-glucosidase inhibition of S. aquifolium which could potentially lead to type 2 diabetes prevention and needs further characterization and *in vivo* clinical studies.

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