Potential antiradical and alpha-glucosidase inhibitors from *Ecklonia maxima* (Osbeck) Papenfuss

Kannan R.R. Rengasamy, Mutalib A. Aderogba, Stephen O. Amoo, Wendy A. Stirk, Johannes Van Staden *

Research Centre for Plant Growth and Development, School of Life Sciences, University of KwaZulu-Natal, Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa

**Abstract**

Alpha-glucosidase inhibitors play a potential role in the treatment of type 2 diabetes by delaying glucose absorption in the small intestine. *Ecklonia maxima*, a brown alga which grows abundantly on the west coast of South Africa, is used to produce alginate, animal feed, nutritional supplements and fertilizer. The crude aqueous methanol extract, four solvent fractions and three phlorotannins: 1,3,5-trihydroxybenzene (phloroglucinol) (1), dibenzo[1,4]dioxine-2,4,7,9-tetraol (2) and hexahydroxyphenoxystibenzene (eckol) (3) isolated from *E. maxima* were evaluated for antiradical and alpha-glucosidase inhibitory activities. All the phlorotannins tested had strong antioxidant activities on DPPH free radicals with EC$_{50}$ values ranging from 0.008 to 0.128 μM. Compounds 2 and 3 demonstrated stronger antioxidant activity and an alpha-glucosidase inhibitory property than positive controls. These results suggest that *E. maxima* could be a natural source of potent antioxidants and alpha-glucosidase inhibitors. This study could facilitate effective utilization of *E. maxima* as an oral antidiabetic drug or functional food ingredient with a promising role in the formulation of medicines and nutrition supplements.

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1. Introduction

Diabetes mellitus (DM) is a complex disorder characterized by hyperglycemia. It is one of the world’s most serious health concerns, developing increasingly with the increasing obesity and advancing age in the general global population. The world prevalence of diabetes among adults (aged 20–79 years) is 6.4%, affecting 285 million adults, in 2010, and is estimated to increase to 7.7%, affecting 439 million adults by 2030 (Shaw, Sicree, & Zimmet, 2010). The global prevalence of diabetes is 6.4%. It varies from 10.2% in the Western Pacific to 3.8% in the African region. However, the African region is expected to experience the highest increase. According to Levitt (2008), 10.8 million people had diabetes in sub-Saharan Africa in 2006, which could rise to 18.7 million by 2025, which is an increase of 80%, exceeding the globally predicted increase of 55%. In South Africa, the number of adults with diabetes was 1.28 million in 2010 and is expected to reach 1.64 million in 2030, with an annual increment of 18,000 (Shaw, Sicree, & Zimmet, 2010).

The disease is primarily classified into insulin dependent DM (type 1 diabetes), non-insulin dependent DM (type 2 diabetes) and gestational diabetes. Type 2 diabetes is responsible for 85–95% of all diabetes in high-income countries and may account for an even higher percentage in low and middle income countries. It may be effectively managed by preventing absorption of carbohydrates after a meal (Ortiz-Andrade et al., 2007). Alpha-glucosidase is the key enzyme involved in intestinal glucose absorption. Alpha-glucosidase inhibitors are very effective in reducing postprandial glucose by suppressing the absorption of glucose and are effective in the treatment and management of hyperglycemia (diabetes) and hyperlipidemia (obesity) (DeMelo, Gomes, & Carvalha, 2006). Synthetic alpha-glucosidase inhibitors such as acarbose, miglitol and voglibose are widely used since the early 1990s for the treatment of patients with type 2 diabetes as oral antidiabetic drugs. However, they also cause various side-effects like flatulence, diarrhea and abdominal discomfort. Therefore, safer natural alpha-glucosidase inhibitors are desired and many potential compounds have been reported from marine algae (Eom et al., 2012; Heo et al., 2009; Kim, Nam, Kurihara, & Kim, 2008).

Chronic hyperglycemia in diabetes causes oxidative stress (Brownlee, 2005) with oxidative stress playing an important role in complications of diabetes which can be managed by antioxidant agents (Dicarli, Janises, Grunberger, & Ager, 2003). Therefore, plants offer a wide range of antioxidants that can be beneficial for the treatment of diabetes (Pietta, 2000). Marine algae represent one of the richest sources of natural antioxidants (Mayer & Hamann, 2002). Many of the phlorotannins purified from brown algae demonstrated strong antioxidant activity comparable to synthetic antioxidants (Kang et al., 2005, 2006). Other beneficial biological activities of brown alga constituents include:
Table 1  
DPPH radical scavenging activity of the crude extracts and different solvent fractions of E. maxima.

<table>
<thead>
<tr>
<th>Test sample</th>
<th>EC50 values (μg/ml)</th>
<th>AAI</th>
<th>R² value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>4.047 ± 0.43 b</td>
<td>0.487 ± 0.005</td>
<td>0.979</td>
</tr>
<tr>
<td>Hexane</td>
<td>85.77 ± 2.17 d</td>
<td>0.230 ± 0.005</td>
<td>0.983</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>58.6 ± 4.06 c</td>
<td>0.339 ± 0.023</td>
<td>0.977</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>7.43 ± 0.37 a</td>
<td>2.665 ± 0.134</td>
<td>0.997</td>
</tr>
<tr>
<td>Butanol</td>
<td>42.99 ± 1.44 b</td>
<td>0.459 ± 0.015</td>
<td>0.992</td>
</tr>
</tbody>
</table>

Effective concentrations are expressed as the mean ± SEM (n = 3). Mean values followed by different letters are significantly different (P < 0.05) based on Duncan's multiple range test.

2. Materials and methods

2.1. Chemicals

The following chemicals were obtained from Sigma–Aldrich: alpha-glucosidase from Saccharomyces cervisiae (EC 3.2.1.20), p-nitrophenyl-α-D-glucopyranoside and acarbose.

2.2. Plant material – collection, authentication

The brown alga, E. maxima was collected by Kelp Products (Pty) Ltd who have a valid license to harvest E. maxima from Kommetjie on the west coast of South Africa. On the day of harvesting, the fronds and stripes were washed, minced and vacuum-packed at the factory. The packed E. maxima was then sent to the Research Centre for Plant Growth and Development, School of Life Sciences, University of KwaZulu-Natal, Pietermaritzburg by air. On the day of arrival the sample was freeze-dried, powdered and the powdered material stored at −10 °C until required for analysis.

2.3. Extraction, isolation and identification of phlorotannins

The extraction, isolation and identification of compounds 1-3 from the ethyl acetate fraction of E. maxima were as previously described (Kannan, Aderogba, Ndhlala, Stirk, & Van Staden, 2013). Isolated phlorotannins were identified as: 1,3,5-trihydroxybenzene (phloroglucinol) (1), dibenzo [1,4] dioxine-2,4,7,9-tetraol (2) and hexahydroxyphenoxydibenzo [1,4] dioxine (eckol) (3).

2.4. Antiradical activity

The free radical scavenging activity was evaluated using the DPPH assay as described by Karioti, Hadjipavlou-Litina, Mensah, Fleischer, and Skaltsa (2004) with modifications as outlined by Fawole et al. (2010). Ascorbic acid and butylated hydroxytoluene (BHT) were used as positive controls. The negative control had methanol in place of the extract or compound. Background solutions with methanol in place of DPPH solution were included for each sample, in order to remove any absorbance due to the sample colour. The determinations were carried out in triplicate. The radical scavenging activity (RSA) was calculated using Eq. (1):

\[
\text{RSA} \% = 1 - \frac{(A_{\text{sample}} - A_{\text{background}}) / A_{\text{control}} \times 100}
\]

where \(A_{\text{sample}}\), \(A_{\text{background}}\) and \(A_{\text{control}}\) are the absorbance values of the sample, background solution and negative control respectively, at 517 nm. The EC50, which is the concentration of the sample required to scavenge 50% of DPPH free radical was determined for each sample. The antioxidant activity index (AAI) for each sample was calculated using Eq. (2) (Scherer & Godoy, 2009):

\[
\text{AAI} = \frac{\text{final DPPH concentration}}{\text{EC50}}
\]

2.5. Alpha-glucosidase inhibitory activity

Alpha-glucosidase inhibitory activity was determined as previously described by Tao, Zhang, Cheng, and Wang (2013) with slight modifications using a 96-well microtiter plate. Briefly, yeast alpha-glucosidase (0.1 Unit/ml) was dissolved in 0.1 M potassium phosphate buffer (pH 6.8), this was used as the enzyme solution. The substrate, 0.375 mM of p-nitrophenyl-α-D-glucopyranoside (pNPG) was prepared in the same buffer (pH 6.8). The samples: extract, solvent fractions and isolated compounds were individually dissolved in dimethylsulfoxide (DMSO). Each sample (20 μl) and enzyme solution (20 μl) were mixed in the microtiter plate. The reaction was initiated by adding 40 μl substrate. The reaction mixture was incubated at 37 °C for 40 min. After incubation, 80 μl

Table 2  
DPPH radical scavenging activity of phlorotannins isolated from E. maxima.

<table>
<thead>
<tr>
<th>Test sample</th>
<th>IC50 values (μg/ml)</th>
<th>AAI</th>
<th>R² value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phloroglucinol</td>
<td>0.128 ± 0.001 d</td>
<td>390.554 ± 2.469</td>
<td>0.994</td>
</tr>
<tr>
<td>Dibenz [1,4]dioxine-2,4,7,9-tetraol</td>
<td>0.012 ± 0.001 b</td>
<td>4037.183 ± 251.20</td>
<td>0.988</td>
</tr>
<tr>
<td>Eckol</td>
<td>0.008 ± 0.0004 c</td>
<td>6095.914 ± 289.3</td>
<td>0.970</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>0.011 ± 0.0001 ab</td>
<td>4356.12 ± 39.625</td>
<td>0.986</td>
</tr>
<tr>
<td>BHT</td>
<td>0.074 ± 0.002 c</td>
<td>676.79 ± 19.177</td>
<td>0.943</td>
</tr>
</tbody>
</table>

Effective concentrations are expressed as the mean ± SEM (n = 3). Mean values followed by different letters are significantly different (P < 0.05) based on Duncan's multiple range test. AAI = Antioxidant activity index.

Table 3  
Alpha-glucosidase inhibitory activities of the crude extracts and different solvent fractions of E. maxima.

<table>
<thead>
<tr>
<th>Test sample</th>
<th>IC50 values (μg/ml)</th>
<th>R² value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>2.192 ± 0.511 a</td>
<td>0.956</td>
</tr>
<tr>
<td>Hexane</td>
<td>3.911 ± 0.431 a</td>
<td>0.964</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>2.513 ± 0.07 a</td>
<td>0.993</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>3.732 ± 0.63 a</td>
<td>0.989</td>
</tr>
<tr>
<td>Butanol</td>
<td>31.136 ± 1.57 b</td>
<td>0.916</td>
</tr>
</tbody>
</table>

Inhibitions concentrations are expressed as the mean ± SEM (n = 3). Mean values followed by different letters are significantly different (P < 0.05) based on Duncan's multiple range test.
(0.2 M) sodium carbonate in 0.1 M potassium phosphate buffer (pH 6.8) was added to each well to quench the reaction. The amount of p-nitrophenol (pNP) released was quantified using an Opsyx MR 96-well microplate reader at 405 nm. The control experiment contained the same reaction mixture, but the sample solution was replaced with the same volume of phosphate buffer. Acarbose dissolved in DMSO, was used as a positive control. The determinations were carried out in triplicate. The percentage inhibition (%) was calculated by using the following Eq. (3):

\[
\% \text{ Inhibition} = \left( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100
\]

where \( A_{\text{control}} \) is the absorbance of the control and \( A_{\text{sample}} \) is the absorbance of the sample. The IC50, which is the concentration of the sample required to inhibit the enzyme was determined for each sample.

2.6. Data analysis

Regression analysis for calculating IC50 and EC50 values was done using GraphPad Prism software (version 4.03). A one-way analysis of variance followed by separation of mean values by Duncan's multiple range test was done using SPSS software (version 10.0).

3. Results and discussion

Oxidative stress plays an important role in initiating ß-cell damage and insulin resistance (Dong, Li, Zhu, Liu, & Huang, 2012). Antioxidants may prevent the progressive impairment of pancreatic ß-cell function and thus reduce the occurrence of type 2 diabetes (Song, Manson, Buring, Sesso, & Liu, 2005). It was reported that several phenolic compounds with alpha-glucosidase activity also had moderate antioxidant activity (Bhandari, Jong-Anurakkun, Hong, & Kawabata, 2008; Kwon, Apostolidis, & Shetty, 2008; Shobana, Sreerama, & Malleshi, 2009). Hence, the antioxidant activity of the crude extract (20% aqueous methanol), four solvent fractions (hexane, dichloromethane, ethyl acetate and butanol) and isolated compounds from \( E. \) maxima was evaluated by measuring their ability to scavenge DPPH radicals. The EC50 values of the crude extract and solvent fractions ranged from 7.43 to 85.77 µg/ml (Table 1). The ethyl acetate fraction exhibited most prominent DPPH radical scavenging activity with a significant very low EC50 value (\( P < 0.05 \)) and AAI of 2.665. AAI > 2.0 is classified as very strong antioxidant activity and < 0.5 as poor by Scherer and Godoy (2009). All the phlorotannins tested had strong antioxidant activities on DPPH radicals with EC50 values ranging from 0.008 to 0.128 µM (Table 2). In addition, the EC50 value of eckol was 0.008 µM indicating a stronger activity than that of the positive controls, ascorbic acid and BHT used in this study (Table 2).

Effective control of postprandial hyperglycemia is important in early intervention and prevention of diabetic complications for type 2 diabetes management (Ratner, 2001). Recently the pressure to develop new drugs for type 2 diabetes has been stimulated by the worldwide increase in the incidence of this disease (Nathan, 2007). Alpha-glucosidase inhibitors play a major role in the management of hyperglycemia by delaying the postprandial increase of the blood glucose level after a mixed carbohydrate diet (Puls, Keup, Krause, Thomas, & Hoffmeister, 1977). The inhibitory effect of the crude \( E. \) maxima extract and different fractions against alpha-glucosidase was evaluated to access the antidiabetic effects of \( E. \) maxima. The crude extract and fractions exhibited strong inhibitory activity against alpha-glucosidase with IC50 values ranging from 2.19 to 31.14 µg/ml when compared to the positive control, acarbose (Table 3).

Phlorotannins exhibit various beneficial biological activities (Thomas & Kim, 2011). Inhibitory activities of phlorotannins against alpha-glucosidase have not been widely investigated, except reports on Ecklonia stolonifera, Ecklonia cava and Eisenia bicyclis constituents (Eom et al., 2012; Lee et al., 2010; Moon, Nurul Islam, & Ahn, 2011). To the best of our knowledge, there is no previous report on antidiabetic properties of phlorotannins from \( E. \) maxima which are now presented in Table 4. The phloroglucinol derivative dibenzo [1,4] dioxine-2,4,7,9-tetraol (2) and eckol (3) had better inhibitory activity than the positive control, acarbose (Table 4) with phloroglucinol (1) having a lower activity. This result correlated with the previous report, where the phloroglucinol isolated from \( E. \) stolonifera showed less inhibition against alpha-glucosidase (Moon et al., 2011). The molecular size of the phlorotannins and number of hydroxyl groups present in the molecules were found to be important for the strong interaction with the enzyme molecules and consequent inhibition of the enzyme (Moon et al., 2011). The results of the present study are consistent with this report with compounds 2 and 3 demonstrating better inhibition of the enzyme because of greater molecular size and a larger number of hydroxyl groups than phloroglucinol.

4. Conclusions

The results presented in this study are the first information on extracts, fractions and isolated phlorotannins from \( E. \) maxima focusing on antiradical and antidiabetic properties. Dibenzo [1,4] dioxine-2,4,7,9-tetraol (2) and eckol (3) exhibited significant antioxidant activity compared to the positive controls. Also, compounds 2 and 3 were more potent alpha-glucosidase inhibitors than acarbose that was used as a positive control. The results presented in this study showed that \( E. \) maxima which is available in large quantities in South Africa contained phloroglucinol derivatives with high antiradical activity and strong inhibitory activity against alpha-glucosidase. This makes it a promising candidate that could be used for novel natural antiradical agents and alpha-glucosidase inhibitors to treat type 2 diabetes.

Acknowledgements

The authors thank the Claude Leon Foundation, Cape Town and the University of KwaZulu-Natal for support in the form of Post-doctoral Fellowships.

References

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