ANTIDIABETIC POTENTIAL OF MARINE RED ALGA *CHAMPIA PARVULA* (C. AGARDH) BY INHIBITING KEY METABOLIC ENZYMES

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**ABSTRACT**

Alpha amylase and alpha glucosidase are key enzymes of dietary carbohydrates digestion in humans. Inhibitors of these enzymes may be effective in retarding carbohydrate digestion and glucose absorption to suppress postprandial hyperglycemia. In the present study *in vitro* antidiabetic activity of red alga *Champia parvula* (C. Agardh) was evaluated using alpha amylase and alpha glucosidase. The highest percentage of alpha amylase inhibition by crude methanol extract of *Champia parvula* was found to be \((98.50 \pm 0.02 \text{ µg/g})\) at \(900 \text{ µg/mL}\). Similarly, the highest percentage of alpha glucosidase inhibition by crude methanol extract of *Champia parvula* was found to be \((91.48 \pm 0.02 \text{ µg/g})\) at \(900 \text{ µg/mL}\), nevertheless the \(IC_{50}\) value of alpha amylase shows twofold higher than the alpha glucosidase. Thus, *Champia parvula* can be effective for the treatment of diabetes through inhibition of alpha amylase and alpha glucosidase enzymes and leads to development of natural drug in the pharmaceutical field.

**KEYWORDS:** Alpha Amylase, Alpha Glucosidase, Diabetes, *Champia Parvula*.

**1.0 INTRODUCTION**

Marine macro algae have been one of the richest and most potential sources of primary and secondary metabolites and its innovation has significantly increased in the past three decades (Cardozo *et al.*, 2007). Marine algae are widely distributed in the coastal regions of many continents. The extracts of marine algae exhibit biological activities (Mahasneh *et al.*, 1995).
The red algae are important for the industrial and biotechnological uses because they have phycocolloids, the main source of agar alpha-(1,4)-3,6-anhydro-L-galactose and β-(1,3)-D-galactose with little esterification in cell wall (Chaloupka et al., 2010).

Diabetes mellitus (DM) is one of the most common lifestyle diseases. Diabetes mellitus can be classified under following two categories: Type 1 is insulin dependent diabetes mellitus (IDDM), in which the body does not produce insulin. Type 1 diabetes accounts for 5-10% of diabetes (Cardozo et al., 2007). Type 2 is noninsulin-dependent diabetes mellitus (NIDDM), in which the body does not produce enough, or improper use of secreted insulin is the most common form of the disease, accounting for 90-95% of diabetes is nearing epidemic proportions, due to an increased number of elderly people, and a greater prevalence of obesity and sedentary lifestyles.

Alpha amylase inhibitors bind to alpha bond of polysaccharide and prevent break down of polysaccharide in to mono and disaccharide. Alpha glucosidase is a carbohydrate hydrolyzing enzyme that increases the glucose level. Agents inhibiting alpha-glucosidase are effective therapeutic agents for diabetes, bis (2,3,6-tribromo-4,5-dihydroxybenzyl) ether is the good inhibitor of alpha-glucosidase. The present study an attempt was made to evaluate the in vitro antidiabetic activity of marine red alga Champia parvula.

2.0 MATERIALS AND METHODS

2.1 Collection of algae material: Fresh alga materials was collected from the intertidal regions of the Mandapam coast (Latitude. 09° 17.417’N; Longitude. 079° 08.558’E), South East Coast of Tamilnadu during November 2016 and was immediately brought to the laboratory in plastic bags containing water in order to prevent deterioration. The algal sample was identified by standard manual (Desikachary et al., 1990). The collected alga materials were washed thrice in distilled water to remove adhering sand particles, epiphytes and excess of salts. The washed samples were shade dried for one week at room temperature. The excess water was removed with blotting paper, dried at room temperature and used in experiments. The alga material was cut into small pieces and powder by using electric blender.

2.2 Preparation of Algal extract: The dried algal sample was powdered and then were prepared using methanol as solvent and it was soxhlet. The resultant crude extracts were filtered and then concentrated in a rotary evaporator to obtain the crude methanol extract. The crude extracts were weighed and deep frozen (-20°C) until further use.
2.3 Inhibition assay for α-amylase enzyme

Stock solution of starch was prepared for 0.1% where potato starch (0.1 g) mixed vigorously using 16 mM sodium acetate buffer. The amylase enzyme with concentration of 27.5 mg was diluted with 100 ml of distilled water. The reducing sugars were then detected by reagent mixture of sodium potassium tartarate and 3, 5-dinitrosalicylic acid (DNS) (96 mM) using spectrophotometer at 540 nm.

The various concentrations of the algal extract (100 to 900 µg/mL) were added to 1 mL of starch solution and kept for 10 mins. The diluted enzyme was added to the mixture and incubated for 10 mins at 25°C. The enzymatic reaction was stopped by adding 1 mL of DNS reagent mixture and then kept boiling for 5 mins and cooled to room temperature. 10 mL of distilled water was then added to the reaction mixture and optimal density was measured at 540 nm. The inhibition enzyme activity was measured using the extract and dimethyl sulfoxide (DMSO) served as control. Similar experiments were conducted by using Acarbose as the standard drug.

2.4 Inhibition assay for α-glucosidase enzyme

One ml of 2% maltose/sucrose with 0.2 M Tris buffer pH 8.0 was pre-incubated with different concentrations of algal extract for 5 mins at 37°C. One ml of α-glucosidase enzyme was added and incubated for 40 mins at 35°C. The reaction was stopped by addition of 6 N HCl. Then measured by optical density at 540 nm.

\[
\text{% of inhibition} = \frac{OD \text{ value (Control)} - OD \text{ value (Sample)}}{OD \text{ value (Control)}} \times 100
\]

2.5 Statistical analysis

All data were expressed as mean ± standard error (SE). Statistical analyses were performed using one way analysis of variance (ANOVA) by SPSS software 17.0 version. The difference was considered significant when p<0.005.

3.0 RESULTS

In the present study the methanol extract of C. parvula showed inhibition at different concentrations (100 to 900 µg/mL). The crude methanol extract of C. parvula at the concentration of 900 µg/mL exhibited α-amylase inhibitory activity of 98.50% and the lowest activity at 77.82% of inhibition. C. parvula inhibited the activity of α-amylase with an IC₅₀
value of 173 μg/mL, whereas, the positive control Acarbose inhibited the activity of α-amylase with an IC₅₀ value of 73 μg/mL (Table.1).

The methanol extract C. parvula revealed a significant inhibitory action on α-glucosidase enzyme. The crude methanol extract of C. parvula at the concentration of 900 μg/mL exhibited α-glucosidase inhibitory activity of 91.48% and the lowest activity at 62.25% of inhibition. C. parvula inhibited the activity of α-glucosidase with an IC₅₀ value of 81 μg/mL, whereas, the positive control Acarbose inhibited the activity of α-glucosidase with an IC₅₀ value of 57 μg/mL (Table.2).

### Table.1: In vitro alpha-amylase inhibitory activity of methanol extract of C. parvula.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Concentration µg/mL</th>
<th>Percentage of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Champia parvula</td>
</tr>
<tr>
<td>1</td>
<td>100</td>
<td>77.82 ± 0.02</td>
</tr>
<tr>
<td>2</td>
<td>300</td>
<td>86.82 ± 0.02</td>
</tr>
<tr>
<td>3</td>
<td>500</td>
<td>89.10 ± 0.02</td>
</tr>
<tr>
<td>4</td>
<td>700</td>
<td>93.40 ± 0.01</td>
</tr>
<tr>
<td>5</td>
<td>900</td>
<td>98.50 ± 0.02</td>
</tr>
<tr>
<td>6</td>
<td>IC₅₀</td>
<td>173 μg/mL</td>
</tr>
<tr>
<td>P – Value</td>
<td></td>
<td>0.000</td>
</tr>
<tr>
<td>F – Value</td>
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<td>1.513</td>
</tr>
</tbody>
</table>

### Table. 2: In vitro alpha-glucosidase inhibitory activity of methanol extract of C. parvula.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Concentration µg/mL</th>
<th>Percentage of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Champia parvula</td>
</tr>
<tr>
<td>1</td>
<td>100</td>
<td>62.25 ± 0.125</td>
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<tr>
<td>2</td>
<td>300</td>
<td>74.85 ± 0.313</td>
</tr>
<tr>
<td>3</td>
<td>500</td>
<td>80.74 ± 0.024</td>
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<tr>
<td>4</td>
<td>700</td>
<td>86.40 ± 0.020</td>
</tr>
<tr>
<td>5</td>
<td>900</td>
<td>91.48 ± 0.020</td>
</tr>
<tr>
<td>6</td>
<td>IC₅₀</td>
<td>81 μg/mL</td>
</tr>
<tr>
<td>P – Value</td>
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<td>0.000</td>
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<tr>
<td>F – Value</td>
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<td>5.529</td>
</tr>
</tbody>
</table>

### 4.0 DISCUSSION

Seaweeds and their organic extracts pose wide array of bioactive compounds with potential health benefits (Rindi et al., 2012). Marine seaweeds that can reduce postprandial hyperglycemia by inhibiting enzymes such as alpha-amylase and alpha-glucosidase are found to be an effective strategy for the management of diabetes (Etxeberria et al., 2012).
Preventing disease outbreaks or treating the disease with drugs or chemicals attempts these problems. Moreover the cost of the drugs is high and also they cause adverse effect. (Idose et al., 1968). Many bioactive and pharmacologically important compounds such as alginate, carrageen and agar as phycocolloids have been obtained from seaweeds and used in medicine and pharmacy (Siddhanta et al., 1997).

Therefore, in the present study, the in vitro α-amylase inhibitory studies demonstrated that the methanol extract of C. parvula possess α-amylase inhibitory activity. The percentage of inhibition at 100, 300, 500, 700 and 900 µg/mL concentrations showed a concentration dependant reduction of amylase enzyme activity. The α-amylase inhibitory activities vary widely among the tested algae. As can be observed in C. parvula [IC₅₀=173 µg/mL]. The highest inhibitory activities of these extracts are found to be 98.50% at 900 µg/mL concentrations respectively. It is probably due to the fact that at high extract concentrations, there is a conformational change derived from the binding of compounds to the enzyme (Murugesan et al., 2015; Pandithurai et al., 2015; Murugesan et al., 2016 and Mohanapriya et al., 2016). The low percentage inhibition of α-amylase by the extracts of C. parvula is a pointer to the fact that the alga is a mild inhibitor of the enzyme. Similarly, in α-glucosidase inhibitory activity, the highest inhibitory activities of these extracts are found to be 91.48% at 900 µg/mL concentrations respectively. As can be observed in C. parvula [IC₅₀=81 µg/mL]. It is probably due to the facts that at high extract concentrations. That is, the active components in the extract do not complete with the substrate for the active site of the enzyme, whereas the inhibitors bind to a separate site on the enzyme to retard the conversion of substrate to product (Mayer et al., 2012).

The alpha amylase and alpha-glucosidase enzyme inhibitory effect of methanol extract of the marine red alga C. parvula was studied to find out the possible mechanism of its anti-diabetic activity. The extracts from some macro algae such as Rhodomela confervoides, Gracilaria textorii, Plocamium telfairiae, reported for the strong inhibitory activity of alpha–glucosidase. Marine red algal species Pterocladia capillacea have been reported to constitute primarily galactose as the major component in addition to xylose, glucose and glucoronic acid residues (Abdel Fattah et al., 1973). Gracilaria edulis and Syringodium isoetifolium were shown to have significant effect on glucose utilization (Abideen and Sankar, 2015). Similarly, in the present study a potent inhibitory activity of alpha glucosidase and alpha
amylase enzymes. T. glomerulata red alga break down of starch by inhibits the action of alpha glucosidase enzymes (Mohanapriya et al., 2016)

The extracts from some macroalgae such as Rhodomela confervoides, Gracilaria textorti, Plocamium telfairiae, Dictyopteris divaricata, Ulva pertusa and Enteromorpha intestinalis reported for the strong inhibitory activity of alpha-glucosidase (Kim et al., 2010). Most of the marine red and green algae are found to be effectively inhibitors of alpha-glucosidase. The bromophenols also have hypoglycaemic potentials by inhibiting alpha-glucosidase and aldose reductase which was found in some red algae like Rhodomela confervoides, Symphyocladia latiuscula and Polysiphonia urceolata shows significant hypoglycaemic activity (Senthilkumar and Ahmed John 2008; Ragan and Glombitza; Heo et al., 2009; Lee et al., 2010 and Seung-Hong and You-Jin 2013; Murugesan et al., 2016). It is concluded from the present findings that, the methanol extract of marine red alga C. parvula showed significant effect on glucose utilization.

5.0 CONCLUSION
In, conclusion the present studies the antidiabetic potential of marine red alga Champia parvula and in particular suggests that phytoactive compounds may have potential anti diabetic effects through the inhibition of both alpha amylase and alpha glucosidase enzymes. Thus, the marine red alga C. parvula effectively inhibit both alpha-amylase and alpha–glucosidase enzymes in vitro in a dose dependent manner which paves a way for further in vivo antidiabetic properties of these extracts and the identification of active compounds is in progress.

6.0 ACKNOWLEDGEMENT
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7.0 REFERENCES
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