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Research Article

EVALUATION OF ALPHA-AMYLASE AND ALPHA-GLUCOSIDASE INHIBITORY PROPERTIES OF SELECTED SEAWEEDS FROM GULF OF MANNAR

Palanisamy SenthilKumar and Sellappa Sudha*

[1]Molecular Diagnosis and Drug Discovery Laboratory, Department of Biotechnology, School of Life Sciences, Karpagam University, Coimbatore-640 021, Tamilnadu, India

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*Email: sudhasellappa@gmail.com

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 *Gracilaria 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 IC_{50} 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 IC_{50} 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 amylase inhibitory activity.

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by increased blood glucose levels with instability in carbohydrate, fat and protein metabolism[1]. 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[2].

Hyperglycemia defining established diabetes can induce oxidative stress by various mechanisms[3]. Oxidative stress has been shown to have a significant effect in the causation of diabetes as well as diabetes related complications[4]. 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[5]. Plants have always been an excellent source of drugs and many of the currently existing drugs have been derived directly or indirectly from them[6]. Previous study shows that seaweeds are known to contain α-glucosidase inhibitors[7].

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

CHEMICALS

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) [8]. 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 $CaCl_2$. The tubes containing substrate solution were boiled for 5 min and were then incubated at 37ºC 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 (PPA) 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 37ºC 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 4ºC. 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:

$= [(A_c+) – (A_c-)] – [(A_s-A_b)] / [(A_c+) – (A_c-)] \times 100$

Where A_c+, A_c-, A_s and A_b are defined as the absorbance of 100% enzyme activity (only solvent with enzyme), 0% enzyme activity (only solvent without enzyme activity), a test sample (with enzyme) and a blank (a test sample without enzyme), respectively.

α-GLUCOSIDASE ENZYME INHIBITION ASSAY

The α-Glucosidase inhibition was determined using the modified method of Matsui *et.al.* (1996)[9]. The α-glucosidase reaction mixture contained 2.9 mM P-nitrophenyl-α-glucopyranoside (pNPG), 0.25ml of extract (varying concentrations) in DMSO and 0.6 U/ml baker's yeast α-glucosidase in sodium phosphate buffer, pH 6.9. Control tubes contained only DMSO, enzyme and substrate, while in positive controls acarbose replaced the seaweed extract. Mixtures without enzyme, seaweed extract and acarbose served as blanks. The reaction mixtures were incubated at 25ºC for 5 min, after which the reaction was stopped by boiling for 2 min. Absorbance of the resulting p-nitro phenol (pNP) was determined at 405nm using spectrophotometer (UV-Vis spectrophotometer UV-2450 (Shimadzu)) and was considered directly proportional to the activity of the enzyme. Glucosidase activity was determined as percentage of control as follows:

% Glucosidase inhibition = 100% - % activity of test as percentage of control

% Activity of test = corrected A_{405} of test x 100% / A_{405} of controls

In order to eliminate background readings, the absorbance of the extract without substrate and enzyme was subtracted from absorbance of the extract and substrate mixtures as follows:

Corrected A_{405} test samples = A_{405} extract and substrate mixture - A_{405} extract alone

The activity in controls (with α- glucosidase but without inhibitor) was considered to be 100%. Concentrations of extracts resulting in 50% inhibition of enzyme activity (IC_{50} values) were determined graphically.

STATISTICAL ANALYSIS

The statistical analysis was performed using one way analysis of variance (ANOVA). Results are expressed as mean ± SD and n = 3.

RESULTS

INHIBITION OF A-GLUCOSIDASE ACTIVITY

The α-glucosidase inhibitor effectiveness of aqueous extracts of the different seaweed species were compared on the basis of their resulting IC_{50} values *G. edulis* inhibited the activity of α-glucosidase with an IC_{50} 46 µg/ml. *S. polycystum,* extract, with an IC_{50} value of 50 µg/ml was second most active of the species tested. *U. lactuca* with an IC_{50} Value of 53 µg/ml, G. *corticata* IC_{50} Value 87 µg/ml was less active. Acarbose, the positive control used in this study, inhibited the activity of α-glucosidase with an IC_{50} Value estimated at 51 µg/ml (Table 1 and Figure 1).

INHIBITION OF α-AMYLASE ACTIVITY

In the present study four species of marine seaweeds tested, all four species including *U. lactuca, S. polycystum, G. edulis, and G. corticata* were found to possess favorable α-amylase inhibitory effects on starch break down *in vitro*. The α-amylase inhibitor effectiveness of aqueous extracts of the different seaweed species were compared on the basis of their resulting IC_{50} values *S. polycystum* inhibited the activity of α-amylase with an IC_{50} value of 60µg/ml. *U. lactuca* extract, with an IC_{50} value of 67 µg/ml was second most active of the species tested. *G. corticata* with an IC_{50} Value of 82 µg/ml, *G. edulis* IC_{50} Value 83 µg/ml was less active. Acarbose, the positive control used in this study, inhibited the activity of α-amylase with an IC_{50} Value estimated at 64 µg/ml (Table 1 and Figure 2).

DISCUSSION

In diabetes mellitus, control of postprandial plasma glucose level is critical in the early treatment. Inhibition of enzymes involved in the metabolism of carbohydrates is one of the therapeutic approaches for reducing postprandial hyperglycemia[10].

Alpha-glucosidase is a key enzyme in carbohydrate digestion. It catalyzes the hydrolysis of 1, 4-α- glucosidic bonds within carbohydrates with release of α-glucose and promotes the increase of blood glucose level after meal. Alpha-glucosidase inhibitors antagonize the activity of α-glucosidase, thereby delaying intestinal carbohydrate absorption and slowing the sharp rise in blood sugar levels that diabetic patients typically experience after meals[11]. For this reason, α-glucosidase inhibitors, such as acarbose and voglibose, are clinically used as oral antihyperglycemic agents[12-13]. However, they often cause severe gastrointestinal side effects. Therefore, search for new α-glucosidase inhibitors from natural resources has become an attractive approach for the treatment of postprandial hyperglycemia.

Many natural resources have been reported for their anti-diabetic activities in Ayurveda for the treatment of diabetes. However, such resources have not gained much importance as medicines due to the lack of sustained scientific evidence. Kurikara *et. al.,*[14] reported α-glucosidase inhibitory effects of brown and red sea weeds.

In the present study, four sea weeds from the coastal waters of Mandapam, southeast coast of India, were screened for their α-amylase and α-glucosidase inhibitory potential.

The α-glucosidase and α-amylase enzyme 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 textorii* (Suringar) De Toni, *Plocamium telfairiae* Harv., *Dictyopteris divaricata* (Okam.) Okam,*Ulval pertusa* and *Enteromorpha intestinalis* (L.) reported for the strong inhibitory activity of alpha-glucosidase [15]. Similarly, our study reports a potent inhibitory action of *G. edulis* against enzyme α-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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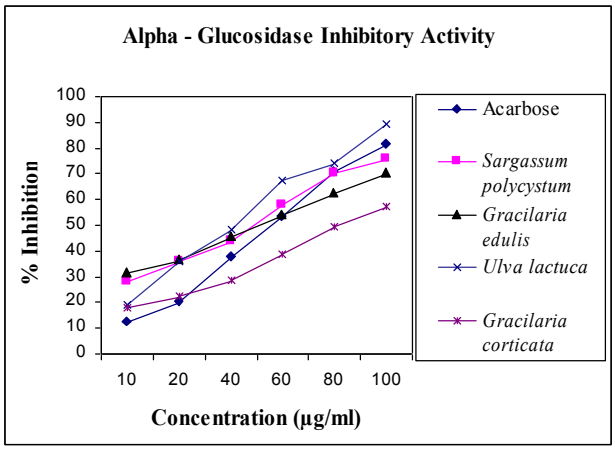

Figure 1: *In vitro* alpha – glucosidase inhibitory activity of seaweeds

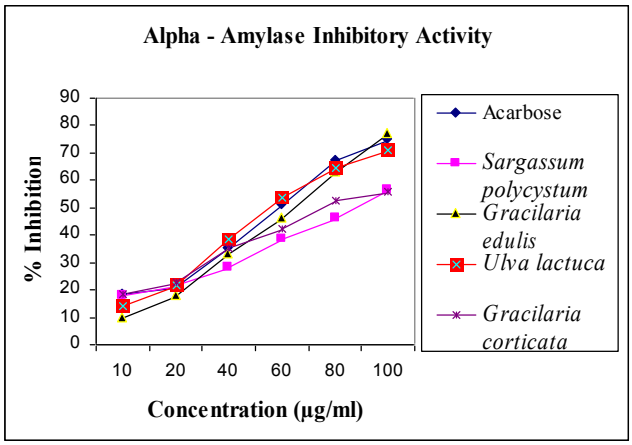

Figure 2: *In vitro* alpha –amylase inhibitory activity of seaweeds

Table 1. IC_{50} value of α-glucosidase and α-amylase aqueous extracts of seaweeds

S.No	Seaweeds	IC_{50} value	
		α-glucosidase inhibition activity	α-amylase inhibition activity
1.	*G. edulis*	46(µg/ml)	83(µg/ml)
2.	*S. polycystum*	50(µg/ml)	60(µg/ml)
3.	*U. lactuca*	53(µg/ml)	67(µg/ml)
4.	*G. corticata*	87(µg/ml)	82(µg/ml)

Results represented the mean of independent triplicate experiments.

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