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Research Article

In vitro α-amylase and α-glucosidase inhibition activity of methanolic extract of marine brown alga *Spatoglossum asperum*

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Abstract

The intent of the present study was to provide an *in vitro* evidence for potential of the marine brown alga against α-amylase and α-glucosidase enzymes. The methanolic extract of brown alga *Spatoglossum asperum* showed significant α-glucosidase inhibitory activity of 96.75 ± 0.03% at the concentration of 900 µg/mL (IC_{50} = 61 µg/mL). Comparably similar effect was observed against α-amylase with 95. 37 ± 0.02% of inhibition (IC_{50} = 55 µg/mL). The above results were compared with that of the standard drug acarbose. From the above results it can be concluded that the algal extract shows an effective antidiabetic activity and indicates that *Spatoglossum asperum* extracts can be considered as a potential source for the management of diabetes mellitus.

1. Introduction

Diabetes mellitus is a metabolic disorder characterized by increased blood glucose levels with instability in carbohydrates, fat and protein metabolism**.** It is a most serious and chronic disease whose incidence rates are increasing obesity and also with aging of the general population of the world. Currently, an estimated 150 million people worldwide have diabetes and that this will increase 220 million by 2010 and 300 million by 2025[1]. Globally, type II diabetes (Non insulin dependent diabetes mellitus) accounts for greater than 90% of the cases [2]. An increased level of glucose in the blood can damage some of the body's systems, such as the blood vessels and the nerves [3]. One therapeutic approach to decrease postprandial hyperglycemia is to retard absorption of glucose via inhibition of carbohydrates hydrolyzing enzymes, such as α-glucosidase and α- amylase.

Evaluation and identification of some new natural molecules with antidiabetic property have become one of the major objectives of present diabetic research. Marine environment is an exceptional reservoir of biologically active products. It is one of the richest sources for floral wealth and diversity [4]. Because of the unusual diversity of chemical structures isolated from marine organisms, there is an intense interest in screening marine natural products for their biomedical potential. A number of reports indicate that seaweeds are still employed in folk medicine for treatment of several diseases. Hence the present study was conducted to evaluate the α-amylase and α-glucosidase inhibitory activity of methanolic extract of *S. asperum*.

2. Materials and Methods

2.1 Preparation of algal material

The marine brown alga *Spatoglossum asperum* was collected from the Mandapam, South East coast of Tamilnadu. The sample was identified by Prof. R. Thevanathan, Professor in Botany, Presidency College, University of Madras, Chennai.

2.2 Algae extracts prepared

The freshly collected samples were soaked and thoroughly cleaned in sea water to remove the sand and salt contents and then shade dried. Dried seaweed was powdered and then soaked in methanol (1:20, w/v) overnight and filtered to collect the methanol fraction. The residue was extracted two more times and the filtrates were combined and concentrated to obtain the crude extract.

2.3 Inhibition of alpha amylase enzyme

A starch solution of 0.1% w/v was prepared by stirring 0.1g of potato starch in 100 mL of 16 mM of sodium acetate buffer. The enzyme solution was prepared by mixing 27.5 mg of alpha amylase in 100 mL of distilled water. The colorimetric reagent was prepared by mixing sodium potassium tartarate solution and 3, 5 di nitro salicylic acid solution 96 mM. Various concentrations of the algal extract (100 to 900 µg/mL) were added to 1 mL of starch solution and left for 10 min. Further the reaction was initiated by the addition of the enzyme solution and allowed to react for 10 min under alkaline condition at 25˚C. Finally the reaction was terminated by adding 1 mL of colorimetric reagent and then incubated in a boiling water bath for 5 min, cooled to room temperature. The reaction mixture was then diluted by adding 10 mL of distilled water and absorbance was measured at 540 nm. Control represent 100% enzyme activity and were conducted in a similar way by replacing extract with DMSO. Similar experiment was conducted with the standard drug Acarbose.

2.4 Inhibition of alpha glucosidase enzyme

The inhibitory activity was determined by incubating a solution of starch substrate (2% w/v maltose or sucrose) 1 mL with 0.2 M Tris buffer pH 8.0 and various concentrations of algal extract for 5 min at 37˚C. The reaction was initiated by adding 1 mL of alpha glucosidase enzyme (IU/mL) to it followed by incubation for 40 min at 35˚C. Then the reaction was terminated by the addition of 2 mL of 6N HCl. The intensity of colour was measured at 540 nm. Control experiment was done by replacing the extract with DMSO and the standard drug Acarbose. Percentage of inhibition was calculated by using the following formulae,

$$\% \text{ of inhibition} = \frac{(\text{OD value of control} - \text{OD value of samples})}{\text{OD value of control}} \times 100$$

3. Results

3.1 Inhibition assay for α-amylase enzyme

In the present study, methanolic extract of marine brown alga *Spatoglossum asperum* was assessed for inhibition of α-amylase effects on starch break down *in vitro* and showed potent α-amylase inhibitory activity. The crude methanolic extract of *S. asperum* at the concentration of 900 µg/mL exhibited 95.37 ± 0.02% of inhibition (Table.1). The inhibitory activity of methanolic extracts of the brown alga was explored on the basis of their resulting IC_{50} values. There was a dose-dependent increase in the percentage inhibitory activity against α-amylase enzyme. *S. asperum* inhibited the activity of α-amylase with an IC_{50} value of 55 µg/mL. Whereas, the positive control Acarbose used in this study, shows an IC_{50} value of 73 µg/mL. (Table.1 and Fig.1).

Table 1: Alpha amylase inhibition activity (%) of Acarbose and methanolic extract of *S.asperum*

S. No	Concentration (µg/mL)	Acarbose	*S.asperum*
1	100	68.54 ± 0.04	91.31 ± 0.02
2	300	76.19 ± 0.04	92.02 ± 0.01
3	500	83.82 ± 0.03	93.46 ± 0.03
4	700	85.99 ± 0.03	94.57 ± 0.02
5	900	86.34 ± 0.03	95.37 ± 0.02
6	IC_{50}	73 µg/mL	55 µg/mL
P – Value		0.000	0.000
F – Value		1.303000	1.656444

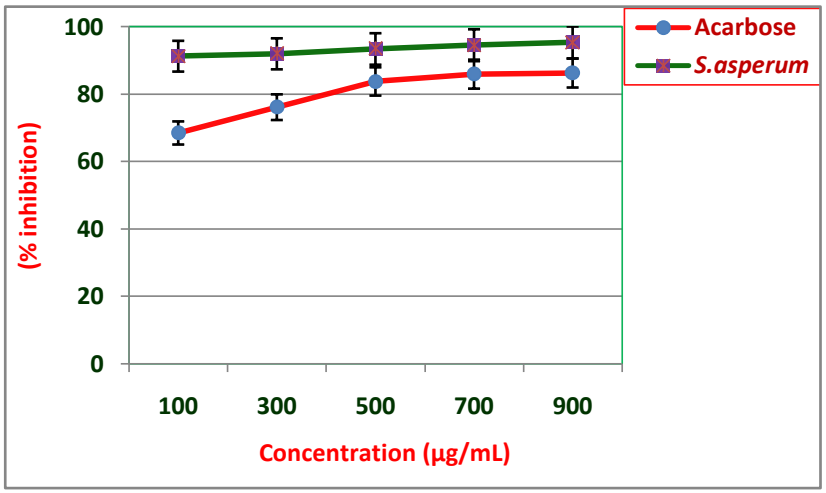

Fig.1: Percentage of α-amylase inhibitory effects of acarbose (standard drug) and methanolic extract of *S.asperum*

3.2 Inhibition assay for α-glucosidase enzyme

The methanolic extract revealed a significant inhibitory action on α-glucosidase enzyme. The crude methanolic extract of *S. asperum* at the concentration of 900 µg/mL exhibited α-glucosidase inhibitory activity of 96.75% (Table.2). *S. asperum* inhibited the activity of α-glucosidase with an IC_{50} value of 61 µg/mL and it was comparably higher than that of standard drug Acarbose (57 µg/mL) (Table.2; Fig.2).

Table 2: Alpha-glucosidase inhibition activity (%) of Acarbose and methanolic extract of *S. asperum*

S. No	Concentration (µg/mL)	Acarbose	*S.asperum*
1	100	88.82 ± 0.02	82.75 ± 0.02
2	300	90.25 ± 0.01	93.05 ± 0.03
3	500	91.63 ± 0.01	95.56 ± 0.02
4	700	93.45 ± 0.02	95.84 ± 0.03
5	900	94.23 ± 0.03	96.75 ± 0.03
6	IC_{50}	57µg/mL	61 µg/mL
P –Value		0.000	0.000
F –Value		3.056444	1.283555

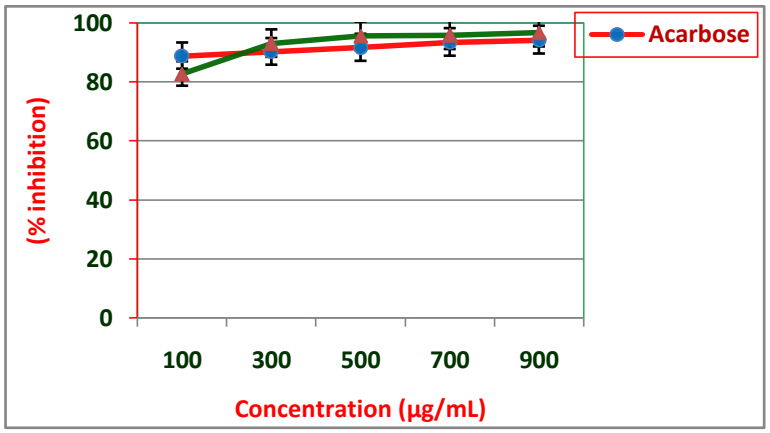

Fig 2: Percentage of α-glucosidase inhibitory effects of acarbose (standard drug) and methanolic extracts of *S. asperum*

4. Discussion

The inhibitory effects of methanolic extract of *S. asperum* on the α-amylase and α-glucosidase activity may be attributed to the presence of phytochemicals such as flavonoids, tannins and saponins. Previous studies attributed the medicinal property of the aqueous extract of *Blighia sapida* to the presence of saponins [5-6]. Newly reported group of α-glucosidase inhibitors is the marine natural bromophenols [7-10], which are usually isolated from the marine algae.

Inhibition of alpha amylase enzyme activity has been noted for phlorotanins from brown algae, *Ecklonia cava* and the IC_{50} value of the most effective component, decal, was calculated to be around 90 µg/mL. However phlorotannins from *Ascophyllum* have a different composition than *Ecklonia* [11-12] and it should be noted that the crude phlorotannin rich fraction from *Ecklonia* was considerably more effective in inhibiting alpha amylase than the most inhibitory single component [13].

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 [14]. Similarly, the present study reports a potent inhibitory action of *S. asperum* against enzyme α-amylase and α-glucosidase enzyme when compared with Acarbose.

Phytochemicals are natural bioactive compounds from biological sources with general benefits to human health. These secondary metabolites may be act as hypolipemic and hypoglycemic agents which helps in reducing blood pressure and regulate cholesterol levels [15]. They are considerable pharmaceutical importance since they are used as drugs for the treatment of several diseases known to man [16]. Marine organisms are potentially prolific sources of highly bioactive secondary metabolites that might represent useful leads in the development of new pharmaceutical agents [17-19].

5. Conclusion

From the discussion above, it is concluded that the results of the present study have unveil the blood glucose lowering activity in the marine brown alga *S. asperum* extract, will be further fractionated in order to search for the antidiabetic ingredients and also comprehensive pharmacological investigations are needed to elucidate the exact mechanism of the antihyperglycemic effect of *S. asperum*.

References

[1] Li. Y., Wen, S., Koda, B.P., Peng, G., Li, G.Q., Yamahara, J., Roufogalis, B.D. *Punica granatum* flower extract, a potent α-glucosidase inhibitor, improves postprandial hyperglycemia in Zucker diabetic fatty rates. *J. Ethanopharmocol*. 2005; 99: 239-244.

[2] Tewari, A, K et al. Diabetes mellitus and multiple therapeutic approaches of phytochemicals: Present status and future prospects. *Curr. Sci*.2002; 83 (1): 30-38.

[3] Matsui, T., Tanaka, T., Tamura, S., Toshima, A., Miyata, Y. & Tanaka, K. Alpha-glucosidase inhibitory profile of catechins and theaflavins. *Journal of Agricultural & Food Chemistry, 2007;* 55 (1): 99-105.

[4] Brad, K Carte. Biomedical potential of Marine Natural Products. *BioSciences*. 1996; 46 (4): 271-286.

[5] Abolaji, O. A., Adebayo, A. H and Odesanmi, O. S. Nutritional qualities of three medicinal plant parts (*Xylopia aethiopica, Blighia sapida* and *Parinari polyandra*) commonly used by Pregnant Women in the Western Part of Nigeria. *Pakistan Journal of Nutrition* 2007; 6(6): 665-668.

[6] Aderinola, O. A., Farinu, G. O., Akinlade, J. A., Olayeni, T. B., Ojebiyi, O. O and Ogunniyi, P. O. Nutritional potential of *Blighia sapida* Koenig (Ackee) leaves as a dry season feed resource for West Africa dwarf Goats in the derived savanna zone of Nigeria. *Livestock Res. & Rural Dev*., 2007; 19(6): 1-5.

[7] Kurihara, H., Mitani, T., Kawabata, J and Takahashi, K. Inhibitory potencies of bromophenols from Rhodomelaceae algae against a-glucosidase activity. *Fish Sci*: 1999; 65: 300–303.

[8] Kurihara, H., Mitani, T., Kawabata, J and Takahashi, K. Two new bromophenols from red alga *Odonthalia cormbifera*. *J Nat Prod*; 1999; 62: 882–884.

[9] Kim, K.Y., Nam, K.A., Kurihara, H and Kim, S.M. Potent α-glucosidase inhibitors purified from the red alga *Grateloupia elliptica Phytochemistry*; 2008; 69: 2820–2825.

[10] Kim, K.Y., Nguyen, T.H., Kurihara, H and Kim, S.M. Alpha-glucosidase inhibitory activity of bromophenol purified from the red alga *Polyopes lancifolia. J Food Sci*, 2010; 75(5):45-50.

[11] Koivikki. R., Loponen. J., Pihlaja, K., and Jormalainen, V. High performance of liquid chromatographic analysis of phlorotannins from the brown alga *Fucus vesiculosus. Phytochemical analysis*, 2007; 18, 326-332.

[12] Ragan, M. A., and Glombitza, K.W. Phlorotannins, brown algal polyphenols. *Progress in Phycological Research*, 1986; 4, 129-147.

[13] Lee, S.H., Li, Y., Karadeniz, F., Kim, M.M and Kim, S.K. α-Glucosidase and αs-amylase inhibitory activities of phloroglucinol derivatives from edible marine brown alga, *Ecklonia cava. Journal of the Science of Food and*

Agriculture, 2008; 89: 1552–1558.

[14] Xiancui, L., Rough, N., Xiao, F., Lijun, H and Lixin, Z. Macroalgae as source of α-glucosidase inhibitors *Chin. J. Oceanol. Limmol*. 2005; 23: 354-356.

[15] Krishnamoorthy, V. Edible seaweeds. In: Souvenir, Natl, Natl Symposium mar. Plants. Their chemistry and Utilization. 2005; 1-4pp.

[16] Okwa, D.E., Josiah, C. Evaluation of the chemical composition of two Nigerian medicinal plants. *Afr. J. Biotechnol.* 2006; 5(4): 357-361.

[17] Iwamoto, C., Minoura, K., Hagishita, S., Nomoto, K., Numata, A. Penostatins F–I novel cytotoxic metabolites from a Penicillium species from an *Enteromorpha* marine alga. *J. Chem Soc. Perkin Trans*. 1998; I 3: 449–456.

[18] Iwamoto, C., Minoura, K., Hagishita, S., Oka, T., Ohta, T., Hagishita, S., Numata, A. Absolute sterostructures of novel penostatins A–E from a Penicillium species from an *Enteromorpha* marine alga. *Tetrahedron*. 1999; 55: 14353–14368.

[19] Iwamoto, C., Yamada, T., Ito, Y., Minoura, K., Numata, A. Cytotoxic cytochalasans from a Penicillium species separated from a marine alga. *Tetrahedron*. 2001; 57: 2904–2997.