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Original Article

Evaluation of *in vitro* antidiabetic activity of red seaweed *Portieria hornemannii* (Lyngbye) (Silva) and *Spyridia fusiformis* (Wulfen)

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ABSTRACT

In the present study *in vitro* antidiabetic activity of red seaweed *Portieria hornemannii* (Lyngbye) (Silva) and *Spyridia fusiformis* (Wulfen) were evaluated using α-amylase and α-glucosidase for inhibitory activity. The methanol extract of *Spyridia fusiformis* revealed at the concentration of 900 µg/mL, α-amylase shows higher activity (84.72 µg/g) than α-glucosidase (94.75 µg/g). The IC_{50} values wer7e achieved high in *S. fusiformis* at 430 µg/mL in α-amylase and 60 µg/mL in α-glucosidase, nevertheless the IC_{50} value of α-amylase shows a twofold higher than the α-glucosidase. Thus, our results suggest that *P.ornemannii* and *S. fusiformis* could be used as a potent antidiabetic agent.

Keywords: α-amylase; α-glucosidase, Diabetes, *Portieria hornemannii* and *Spyridia fusiformis.*

INTRODUCTION

Diabetes mellitus is a metabolic disorder in which, the body does not produce or properly utilize insulin. In spite of the presence of a series of known antidiabetic medicines in the pharmaceutical market, remedies from marine sources are used with success to treat this disorder. Globally, diabetic cases have exploded in the past two decades at 6% per annum and by the year 2025, 324 million people will be diabetic [1]. Moreover, recently discovered drugs were only ameliorating symptoms and would not cease progression of the disease [2]. According to Sharmanidhi and Garg [3], the synthetic antioxidants for diabetes are suspected to be carcinogenic. Most people in developing countries depend on alternate therapies, including natural resources for their primary health care [4].

Marine algae have been found to have various secondary metabolites. Many marine products that are used for treatment of diabetes throughout the world and there is an increasing demand from patients to use the natural products. Natural sources of drugs from marine algae are used widely, even when their biologically active compounds are unknown, because of their effectiveness, minimal side effects in clinical experience and a relatively low cost. They are one of the less explored sources of pharmacological candidates, and few previous studies have found antidiabetic activities in various marine algae [5-10]. In the present study was to evaluate α-amylase and α-glucosidase inhibitory activity of methanol extract of marine red algae *Portieria hornemannii* and *Spyridia fusiformis.*

MATERIALS AND METHODS

Preparation of algal material: The marine red algae *Portieria hornemannii* (Lyngbye) (Silva) *and Spyridia fusiformis* (Wulfen) were collected from Leepuram, Kanyakumarai, South East Coast of Tamilnadu, India, by hand picking method and were identified by standard manual [11]. The voucher specimens (PCCACL01, and PCCACL2) were deposited at the Herbarium, Department of Botany, Pachaiyappa's College, Chennai. India.

Preparation of Algal extracts: The freshly collected samples were soaked and thoroughly cleaned under running tap water to remove the sand and salt contents. The sample was also gently brushed with a soft brush to remove attached epiphytes, other marine organisms and debris. The cleaned samples were rinsed in distilled water and shade dried. The dried seaweeds were powdered

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and stirred in methanol (1:20, w/v) overnight and filtered to collect the methanol fraction. The residue was extracted twice with methanol and the filtrates were combined followed by concentrated to obtain the crude extract on solid form.

Inhibition assay for α-amylase enzyme: A starch solution of 0.1% w/v was prepared by stirring 0.1 g of potato starch in 100 mL of 16 mM of sodium acetate buffer. The enzyme solution was prepared by mixing 27.5 mg of α-amylase in 100 mL of distilled water. The colorimetric reagent was prepared by mixing sodium potassium tartarate solution and 3, 5-dinitro 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 kept for 10 mins. Further, the reaction is initiated by the addition of the enzyme solution and allowed to react for 10 mins 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 mins followed by cooling to room temperature. The reaction mixture was then diluted after adding 10 mL 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 experiments were conducted with the standard drug.

Inhibition assay for α-glucosidase enzyme: The α-glucosidase 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 extracts for 5 mins at 37˚C. The reaction was initiated by adding 1 ml of α-glucosidase enzyme (U/mL) to it followed by incubation for 40 mins at 35˚C. Then the reaction was terminated by the addition of 2 mL of 6N HCl. Then, the intensity of colour was measured at 540 nm. Control experiment was done by replacing the extract with DMSO and for standard drug Acarbose. Percentage of inhibition was calculated by using the following formulae,

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

RESULTS

Inhibition assay for α-amylase enzyme: In the present study methanol extracts of marine red algae *P. hornemannii* and *S. fusiformis* were assessed for *in vitro* α-amylase inhibitory activity. The crude methanol extract of *S. fusiformis* and *P. hornemannii* at the concentration of 900 µg/mL exhibited α-amylase inhibitory activity of 84.72% and 61.80% (Table.1). The effectiveness of α-amylase inhibitor in the methanol extracts of the red algae was confirmed on the basis of their resulting IC_{50} values. There was a dose-dependent increase in percentage inhibitory activity against α-amylase enzyme. *S. fusiformis* inhibited the activity of α-amylase with IC_{50} value of 175 µg/mL and *P. hornemannii* with IC_{50} value of 430 µg/mL. Acarbose, the positive control used in this study exhibited the IC_{50} value at 630 µg/mL (Table.1).

Inhibition assay for α-glucosidase enzyme: The methanol extract of *P.hornemannii* and *S.fusiformis* revealed a significant inhibitory action on α-glucosidase enzyme. The methanol extract of *S.fusiformis* and *P.hornemannii* at the concentration of 900 µg/mL exhibited, α-glucosidase inhibitory activity of 94.75% and 93.90% respectively (Table.2). The α-glucosidase inhibitor effectiveness of methanol extracts of the red algae was confirmed on the basis of their resulting IC_{50} values. *S. fusiformis* inhibited the activity of α-glucosidase with an IC_{50} value of 57 µg/mL and *P. hornemannii* with an IC_{50} value of 60 µg/mL. The IC_{50} value of standard drug Acarbose against α-glucosidase was found to be 76 µg/mL (Table.2).

DISCUSSION

Diabetes is involved in the development of micro and macro vascular diabetic complications [12]. Inhibition of enzyme activity involved in the metabolism of carbohydrates is one of the therapeutic approaches for reducing postprandial hyperglycemia [13]. Recent advances in understanding the activity of intestinal enzymes (α-amylase and α-glucosidase, as both are important in carbohydrate digestion and glucose absorption) have lead to the development of newer pharmacological agents [14]. The key enzyme in carbohydrate digestion is α-glucosidase. 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.

α-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 [15-17]. The inhibition of enzyme α-amylase activity in the digestive tract of humans is considered to be effective to control diabetes by diminishing the absorption of glucose decomposed from starch by these enzymes [18]. Therefore, effective and nontoxic inhibitors of α-amylase and α-glucosidase have long been sought. The main effect of diabetes is increasing

glycemic level and to reach the normal glycemic level, adequate insulin is required. The other oral hypoglycemic agents like sulfonylureas, biguanides [19], Thiazolidinediones (TZD), alpha-glucosidase inhibitors (AGI) and incretinmimetics (GLP-1, GIP, DPP-4 inhibitors) are also used [20]. However, the continuous administration of these synthetic inhibitors like Acarbose causes any adverse effects, such as diarrhoea, abdominal discomfort, flatulence [21-23], and hepatotoxicity [24]. Therefore, search for new α-glucosidase and α-amylase inhibitors from natural resources has become an attractive approach for the treatment of postprandial hyperglycemia. Seaweeds are known as medicinal source and are rich in secondary metabolites including alkaloids, phenols, flavonoids, saponins, steroids and related active metabolites, which have been extensively used in the drug and pharmaceutical industry [25-28].

Therefore, in the present study, the *in vitro* α-amylase inhibitory studies demonstrated that the methanol extract of *P.hornemannii* and *S.fusiformis* 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 *P. hornemannii* [IC_{50}=430 µg/mL] and *S. fusiformis* [IC_{50}=175 µg/mL). The highest inhibitory activities of these extracts are found to be 84.72% for *S. fusiformis* and 61.80% for *P. hornemannii* 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 [29, 30].

The low percentage inhibition of α-amylase by the extracts of *P.hornemannii* is a pointer to the fact that the algae are a mild inhibitor of the enzyme. That is, the active components in the extract do not compete 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 [31].

The mechanism by which *P. hornemannii* and *S. fusiformis* exerts action may be due to its action on carbohydrate binding regions of α-glucosidase enzyme, α-amylase, endoglucanases that catalyse hydrolysis of the internal α-1, 4 glucosidic linkages in starch and other related polysaccharides have also been targeted for the suppression of postprandial hyperglycemia**.** The inhibitory effects of methanolic extract of *P. hornemannii* and *S. fusiformis* on the α-amylase and α-glucosidase activities, respectively, may be have 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 [32, 33].

One such newly reported group of α-glucosidase inhibitors is the marine natural bromophenols [5, 34, 35], isolated from the marine algae, which competitively inhibits α-glucosidase with an IC_{50} of 0.098 µM [28, 34, 35].

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 α-glucosidase [36]. Similarly, the present study reports a potent inhibitory action of *P. hornemannii* and *S. fusiformis* against enzyme α-amylase and α-glucosidase enzyme and compared with Acarbose. The red algae *Rhodomela ceaeconta* contains bromophenols which have α-glucosidase inhibitory activity [28, 34].

Inhibition of α-amylase has been noted for purifying phlorotannins from another brown alga, *Ecklonia cava* [37]. However, phlorotannins from *Ascophyllum* have a different composition than *Ecklonia* [38, 39]. However, it should also be noted that extracts from *Ulva*, with presumably low phenolic content, also have antidiabetic effects in animal models [40].

Similarly, α-glucosidase and α-amylase inhibitor bromophenol, C_6H_5BrO, is produced by *Polyopes lancifolia* and *Grateloupia elliptica* (red seaweeds). It has been used in the therapy of type II Diabetes mellitus to efficiently control the blood sugar with starch-containing diets. Many other species of seaweeds were reported to produce various other components, which inhibit α-glucosidase and α-amylase activity [41]. Diphlorethohydroxycarmalol isolated from *Ishigeo kamurae* (brown seaweeds) as a potent α-glucosidase and α-amylase inhibitor. It alleviates postprandial hyperglycemia in diabetic mice [41].

CONCLUSION

In the present study it can be concluded that the methanol extract of *P. hornemannii* and *S. fusiformis* effectively inhibited the activity of α-amylase in a non-competitive manner while α-glucosidase in an uncompetitive manner. This inhibitory property of the extracts may be attributed to the presence of phytochemicals such as saponins and flavonoids. However, further study is required to isolate the active enzyme inhibitory component from these algae.

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Conflict of interest statement

We declare that we have no conflict of interest.

Table 1: α-amylase inhibition activity (%) of Acarbose and methanol extract of experimental algae.

S.No	Concentration (µg/mL	Acarbose	*P. hornemannii*	*S.fusiformis*
1	100	25.38	20.07	27.68
2	300	33.34	42.83	74.82
3	500	44.14	55.50	75.77
4	700	56.34	60.02	83.72
5	900	59.68	61.80	84.72
IC_{50}		630 µg/mL	430 µg/mL	175 µg/mL

Table 2: α-glucosidase inhibitory activity (%) of Acarbose and methanol extract of experimental algae

S.No	Concentration (µg/mL)	Acarbose	*P. hornemannii*	*S.fusiformis*
1	100	66.05	87.37	83.96
2	300	74.24	93.07	88.47
3	500	82.50	93.71	94.12
4	700	83.24	93.79	94.57
5	900	84.86	93.90	94.75
IC_{50}		76 µg/mL	60 µg/mL	57 µg/mL

REFERENCES

1. Mohammed A et al. Effects of aqueous extract of *Ganoderma lucidum* on blood glucose levels of normoglycemic and alloxan-induced diabetic wistar rats. J Med Plants Res 2007; 1(2): 34-37.
2. Wolfson and Wendy. Leapin' Lizards: Amylin targets diabetes and obesity via incretins. Chem and Bio 2007; 14:43-23.
3. Sharmanidhi and Garg, V. Antidiabetic and antioxidant potential of ethanolic extract of *Butea monosperma* leaves in alloxan-induced diabetic mice. Indian J Biochem and Biophysics 2009; 46:99-105.
4. Hassan Z et al. Antidiabetic properties and mechanism of action of *Gynura procumbens* water extract in streptozotocin-induced diabetic rats. Molecules 2010; 15:9008–9023.
5. Kim KYet al. Potent α-glucosidase inhibitors purified from the red alga *Grateloupia elliptica* Phytochemistry 2008; 69:2820–2825.
6. Felix Nwosu et al. Anti-proliferative and potential anti-diabetic effects of phenolic-rich extracts from edible marine algae. Food Chemistry 2011; 126:1006–1012.
7. Nagarani N and Kumaraguru AK. Evaluation of Anti-Inflammatory, Antidiabetic, Cytotoxic Activity of Kappaphycus alvarezii. Int J Pharm and Bio Sci 2013; 4(1):921 – 929.
8. Bhesh Raj S et al. *Caulerpa lentillifera* extract ameliorates insulin resistance and regulates glucose metabolism in C57BL/KsJ-db/db mice via PI3K/AKT signaling pathway in myocytes. Journal Transl Med 2015; 13:62.
9. Pandithurai M et al. *In vitro* α-amylase and α-glucosidase inhibition activity of methanolic extract of marine brown alga *Spatoglossum asperum.* Int J Adv Pharm 2015; 4(5):83-87.
10. Murugesan S et al. *In vitro* antidiabetic activity of methanolic extracts of selected marine algae. Eur J Pharm Med Res 2015; 2(6):256-260.
11. Desikachary TV et al. Rhodophyta. Madras Science Foundation, Chennai. 1990:279.
12. Brownlee M et al. Advanced glycosylation end products in tissue and the biochemical basis of diabetic complications. New Eng J Med 1988; 318:1315–1321.
13. Ortiz J et al. Dietary fiber, amino acid, fatty acid and tocopherol contents of the edible seaweeds *Ulva lactuca* and *Durvillaea antarctica*. Food Chem 2006; 99:98–104.
14. Verspohl EJ. Recommended testing in diabetes research. Planta Med 2002; 68:581–590.
15. Kovasu M et al. Impact of acarbose on carotid intima media thickness in patients with newly diagnosed impaired glucose tolerance of mild type 2 diabetes mellitus. A one year prospective randomized open label, parallel group study in Japanese adults with established coronary artery disease. Clin Ther 2010; 32:1610-1617.
16. Frantz S et al. Repetitive postprantial hyperglycemia increases cardiac ischemia reperfusion injury prevention by the inhibitor acarbose. Faseb J 2005; 19:837-842.
17. Shimabukuro M et al. Effects of single administration of acarbose on postprandial glucose excursion and endothelial dysfunction in type 2 diabetic patients a randomized crossover study. J Clin Endocrinal Metab 2000; 91: 837-842.
18. Hara Y and Honda M. The inhibition of α-amylase by tea polyphenols. Agric Biol Chem 1990; 54(8):1939–1945.

19. Schwab S and Diem P. Oral hypoglycaemic agents in 2009. Ther Umsch 2009; 66:677-84.
20. Garber AJ. Incretin-based therapies in the management of type-2 diabetes: rationale and reality in a managed care setting. Am J Manag Care 2010; 16 (7):187-94.
21. Campbell LK et al. Miglitol: Assessment of its role in the treatment of patients with diabetes mellitus. Ann Pharmacother 2000; 34:1291–1301.
22. Krentz AJ and Bailey CJ. Oral antidiabetic agents. Drugs 2005; 65(3):385-411.
23. Nathan DM et al. Management of hyperglycaemia in type 2 diabetes: a consensus algorithm for the initiation and adjustment of therapy: Aconsensus statement from the American Diabetes Association and the European Association for the Study of Diabetes. Diabetologia 2006; 49: 1711-1721.
24. Hsiao SH et al. Hepatotoxicity associated with acarbose therapy. Ann Pharmacother 2006; 40:151–154.
25. Vasanthi H et al. Free radical scavenging and antioxidant activity of a red alga *Acanthophora spicifera* – Relation to its chemical composition. Seaweed Res Utiln 2006; 28(1):119-125.
26. Krishnamurthy V. Edible seaweeds. In: Souvenir, Natl. Symp Mari Plants Their Chemistry and Utilization. 2005; 1-4.
27. Matsui T et al. Alpha-glucosidase inhibitory profile of catechins and *Thea flavins.* J Agric Food Chem 2007; 55:99–105.
28. Kurihara H et al. Inhibitory potencies of bromophenols from Rhodomelaceae algae against a-glucosidase activity. Fish Sci 1999; 65:300–303.
29. Andrade-Cetto A et al. Alfa glucosidase -inhibiting activity of some Mexican plants used in the treatment of type 2 diabetes. J Ethnopharmacolgy 2008; 116:27-32.
30. Kim JS et al. Inhibition of alpha-glucosidase and amylase by luteolin, a flavonoid. Biosci Biotechnol Biochem 2000; 64:2458-2461.
31. Mayur B et al. Antioxidant and α- glucosidase inhibitory properties of *Carpesium abrotanoides* L. J Med Plant Res 2010; 4(15):1547-1553.
32. Abolaji OA et al. 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. Pak J Nutr 2007; 6(6):665-668.
33. Aderinola OA et al. 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.
34. Kurihara H et al. Two new bromophenols from red alga *Odonthalia cormbifera*. J Nat Prod 1999; 62: 882–884.
35. Kim KY et al. Alpha-glucosidase inhibitory activity of bromophenol purified from the red alga *Polyopes lancifolia.* J Food Sci 2010; 75(5):45-50.
36. Xiancui L et al. Macroalgae as source of α-glucosidase inhibitors. Chin J Oceanol Limmol 2005; 23:354-356.
37. Lee SH et al. α-Glucosidase and α-amylase inhibitory activities of phloroglucinol derivatives from edible marine brown alga, *Ecklonia cava.* J Sci Food Agric 2008; 89: 1552–1558.
38. Koivikki R et al. High-performance liquid chromatographic analysis of phlorotannins from the brown alga *Fucus vesiculous.* Phytochem Ana 2007; 18:326–332.
39. Ragan MA and Glombitza KW. Phlorotannins, brown algal polyphenols. In: Hellebustand, J.A., Craigie, J.S. (Eds.), Handbook of Phycological Methods, vol. II. Cambridge University Press, Cambridge, 1986; pp.129–241.
40. Celikler S et al. Anti-hyperglycemic and antigenotoxic potential of *Ulva rigida* ethanolic extract in the experimental diabetes mellitus. Food Chem Toxic 2009; 47:1837–1840.
41. Kim AR et al. Isolation and identification of phlorotannins from *Ecklonia stolonifera* with antioxidant and anti-inflammatory properties. J Agric Food Chem 2009; 57:3483–3489.