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Research Article

In Vitro α-Amylase and α-Glucosidase Inhibitory Activity of Methanol Extract of *Tolypiocladia glomerulata* (C. Agardh) F. Schmitz

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Abstract: The present study is to evaluate the inhibitory activity of α-amylase and α-glucosidase enzymes in the methanol extracts of marine red alga *Tolypiocladia glomerulata*. The methanol extract of red alga *T. glomerulata* showed concentration dependent α-glucosidase inhibitory and with achieved maximum activity of 87.08 ± 0.01% at a concentration of 900 µg/mL in a manner IC_{50} = 608 µg/mL. Similarly the dose dependent effect was observed against α-amylase enzyme with 81.25 ± 0.02% of inhibition at 900 µg/mL (IC_{50} = 543 µg/mL). The above results were compared with that of the standard drug Acarbose. It can be concluded that the methanol extracts of red alga *T. glomerulata* exert an effective antidiabetic activity and considered as a potential candidate for the management of diabetes mellitus.
Keywords: α-amylase, α-glucosidase, antidiabetic activity, *Tolypiocladia glomerulata*.

INTRODUCTION

Diabetes mellitus is a syndrome characterized by chronic hyperglycemia, due to relative deficiency in effectiveness of circulating insulin secretion. The high consumption of carbohydrate and sucrose rich food is one of the main causes of non-insulin dependent diabetes mellitus (type II diabetes). Hydrolysis of dietary starch are the major source of glucose in the blood, α-amylase and α-glucosidase being the key enzymes involved in starch break down and intestinal absorption, respectively. It is believed that inhibition of these enzymes can significantly decrease the postprandial increase of blood glucose level after a mixed carbohydrate diet and therefore, it can be an important strategy in the management of hyperglycemia linked to type II diabetes [1].

Type II diabetes accounts for more than 90% of the cases in the world [2]. The World Health Organization (WHO) has estimated that there are approximately 350 million people who suffer from diabetes and this may double by 2030 when diabetes may become the seventh most prevalent cause of death [3]. Some drugs, such as miglitol and voglibose, are effective only on α-glucosidase [4], but acarbose provides inhibition of both glucosidase and amylase [5]. The side-effects such as abdominal discomfort, flatulence and diarrheoa caused by these drugs could stop them from being used and reduce treatment effectiveness. However, the urge for searching novel alternative medicine from natural resources always being a interesting task for researchers. Result, there has been interest in research to discover natural inhibitory components [6].

There has been a substantial drive to examine edible seaweeds for such components, with a focus on α-amylase and α-glucosidase inhibitors [7]. Seaweeds and their organic extracts are known to contain a wide array of bioactive substances with diverse health benefits [8]. Moreover, they are considered to be good sources of polyphenols and have been shown to exhibit antidiabetic properties through inhibition of carbohydrate-hydrolysing enzymes [9-16]. In the present study, the α-amylase and α-glucosidase inhibitory activity of methanol extract of the red alga *Tolypiocladia glomerulata* was evaluvated.

MATERIALS AND METHODS

Collection, Identification And Processing Of Seaweed

The red seaweed *Tolypiocladia glomerulata* was collected by hand picking from coastal area of Mandapam, Tamil Nadu, (south east coast)India. The collected seaweeds were washed with seawater and with fresh water. The washed seaweeds were shade dried at room temperature until reaching a constant weight and then grounded using a laboratory blender. This powder was used to obtain the extract by using methanol as a solvent. The yield of methanolic extract obtained was *Tolypiocladia glomerulata*.The sample was identified by Dr.M Baluswami,(Retd.) Madras Christian College, Chennai, India. The voucher specimens (PCCACL11)

were deposited at the Herbarium in Department of Botany, Pachaiyappa's College, Chennai.

In Vitro Methods Employed In Antidiabetic Studies

Inhibition Of α-Amylase Enzyme Assay

A total of 500 µL of test samples and standard drug (100-900 µg/mL) was added to 500 µL of 0.20 mM phosphate buffer (pH 6.9) containing α-amylase (0.5 mg/mL) solution and were incubated at 25°C for 10 min. After these, 500 µL of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube. The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 1.0 mL of 3, 5 dinitrosalicylic acid colour reagent. The test tubes were then incubated in a boiling water bath for 5 min, cooled to room temperature. The reaction mixture was then diluted after adding 10 mL distilled water and absorbance was measured at 540 nm. Controls represent 100% enzyme activity and were conducted in a similar way by replacing extract with solvent [17-18]. All assays were carried out in triplicate and values were presented in percentage of inhibition. The activity of α -amylase was calculated as follows:

$$\text{Percentage of inhibition} = \frac{\text{Absorbance of control - Absorbance of sample treated with extract}}{\text{Absorbance of control}} \times 100$$

Inhibition Of α-Glucosidase Enzyme Assay

The inhibitory activity on **α-** glucosidase was determined by incubating a solution of starch substrate (2% w/v maltose or sucrose) with 1mL 0.2 M Tris buffer (pH 8.0) containing concentrations of algal extract for 5 min at 37°C. The reaction was initiated by adding 1mL of α-glucosidase enzyme to it followed by incubation at 37°C for 10 min. Then, the reaction mixture was heated for 2 min in boiling water bath to stop the reaction. The amount of liberated glucose is measured by the glucose oxidase peroxidase method [19-21]. All assays were carried out in triplicate and values were presented in percentage of inhibition.

Statistical Analysis

The data was statistically analyzed by one way ANOVA using SPSS.17.0. The difference was considered significant when $p<0.005$. All the values were expressed as mean ± standard deviation (S.D.).

RESULTS AND DISCUSSION

Evaluation Of *In Vitro* α-Amylase Inhibitory Activity

In the present study the methanol extract of the red alga *T. glomerulata* showed 20.81 ± 0.01% of α-amylase inhibition at a concentration of 100 µg/mL and 81.25 ± 0.02% inhibition at the highest concentration of 900 µg/mL, it was nearly equal to that of the standard drug acarbose (85.34 ± 0.03%). There was a dose-dependent increase in the percentage inhibitory activity against α-amylase enzyme. At high concentration 900 µg/mL of the extract showed 90% efficiency with respect to the standard drug Acarbose. The IC_{50} value of standard drug Acarbose was found to be 73 µg/mL, whereas, the methanol extract of the red alga *T. glomerulata* it was 543 µg/mL (Table.1). The result of the present study was well coincided with the report of [22] in the algae *Chondrococcus hornemannii* and *Gracillaria gracillis.* Whereas, the present study showed lesser activity of α-amylase inhibition when compared to the methanol extract of *Grateloupia lithophila* [16]. The IC_{50} value of the *T. glomerulata* (543 µg/mL) was higher than that of the red *Gracillaria edulis* (83 µg/mL) and *Gracillaria corticata* (82 µg/mL) [23].

Table 1: α-amylase inhibitory activity of methanol extract of *T. glomerulata*

S.No	Concentration (µg/mL)	Percentage of inhibition	
		T. glomerulata	**Acarbose**
1	100	20.81 ± 0.01	69.54 ± 0.04
2	300	27.05 ± 0.02	75.19 ± 0.04
3	500	41.63 ± 0.02	83.84 ± 0.03
4	700	64.57 ± 0.02	84.91 ± 0.03
5	900	81.25 ± 0.02	85.34 ± 0.03
6	IC_{50}	543 µg/mL	73 µg/mL
F–Value		4.169666	1.303555
P–Value		0.000	0.000

Lack of insulin affects the metabolism of carbohydrates, proteins, fats and causes significant disturbance of water and electrolyte homeostasis [24]. Pharmacological properties of α-glucosidase inhibitors such as acarbose that can inhibit pancreatic α-amylase revealed that the complications of diabetes mellitus such as onset of renal, retinal and neurological changes and the development of ischaemic myocardial lesions are prevented delayed [25]. Long-term day-to-day management of diabetes, with acarbose is well tolerated and can improve glycemic control as monotherapy, as well as in combination therapy [26]. The major adverse

effects of acarbose are abdominal distention, flatulence, meteorism and mild diarrheoa [27]. The search for new pharmacologically active agents are Safer, specific and effective hypoglycemic agents from natural sources can lead to the discovery of potent and specific inhibitors for α-amylase has continued to be an important area of investigation. [28]. Organic solvent extracts from seaweeds offer a great potential for the discovery of new antidiabetic drugs [27]. In the present study, antidiabetic activity of methanol extracts of *T.glomerulata* was extensively studied in an attempt to screen for potential inhibitors of α-amylase with antioxidant and free radical scavenging activity.

The marine red algae commonly evidenced for highest inhibitory activity against α-amylase than that of the commercial carbohydrate digestive enzyme inhibitor, acarbose [29]. Polyphenolic compounds and as flavonoids such as phlorotannins from marine algae are known to be associated with a variety of proteins to form complexes. The studies of Kim *et al*., [30] have demonstrated that the hydroxyl groups in polyphenolic compounds, have an important role in promoting inhibitory activity.

Evaluation Of *In Vitro* A -Glucosidase Inhibitory Activity

The methanol extract of *T. glomerulata* revealed a significant inhibitory action on α-glucosidase enzyme. The percentage of inhibition was 15.42 ± 0.01% at the concentration of 100 µg/mL and 87.08 ± 0.01% of inhibition were observed at the concentration of 900 µg/mL. The methanol extract of *T. glomerulata* showed a concentration dependent increase in percentage inhibition. The percentage inhibition was lesser when compared to the standard drug acarbose (94.27 ± 0.03%). The IC_{50} value of standard drug acarbose against α-glucosidase was found to be 57µg/mL, methanol extract of *T. glomerulata* whereas, it was found to be 608 µg/mL for the (Table.2). The IC_{50} value of the *T. glomerulata* (608 µg/mL) was higher than that of the red algae *Gracillaria corticata* (87µg/mL) and *Gracillaria edulis* (46µg/mL), [23]. Although the extracts showed lesser activity than acabose, the bioactive compounds from natural sources could be safer to use as alternate medicine for managing diabetes.

Table 2: α-glucosidase inhibitory activity of methanol extract of *T. glomerulata*

S.No	Concentration (µg/mL)	Percentage of inhibition	
		T. glomerulata	**Acarbose**
1	100	15.42 ± 0.01	87.82 ± 0.02
2	300	24.42 ± 0.00	89.25 ± 0.01
3	500	41.17 ± 0.01	91.63 ± 0.01
4	700	61.24 ± 0.01	93.46 ± 0.02
5	900	87.08 ± 0.01	94.27 ± 0.03
6	IC_{50}	608 µg/mL	57 µg/mL
F–Value		2.213777	3.056444
P–Value		0.000	0.000

The present study showed the higher activity of α-glucosidase inhibition when compared to the red algae, *Symphyocladia latiuscula*, *Odonthalia corymbifera* and *Polysiphonia morrowii* [31], *Grateloupia eliptica* [30], *Grateloupia lithophila* [16]. The results suggest that the methanol extract of *T. glomerulata* efficiently inhibits α-glucosidase enzymes by *in vitro*. Acarbose and miglitol are competitive inhibitors of α-glucosidase retards the digestion of carbohydrates and reduces absorption of starch and disaccharides [32]. Hence, one of the therapeutic approaches for reducing postprandial blood glucose levels in patient with diabetes mellitus is to prevent absorption of carbohydrate after food intake. Inhibition of these enzymes (α-amylase and α-glucosidase) reduced the high postprandial blood glucose peaks in diabetes [33].

Research into the natural products and drug development of algae results in the isolation of over 15,000 novel compounds [34] such as polysaccharides, iodine organic products, macro and micro elements, vitamins and unsaturated fatty acids [35]. Recent studies have demonstrated that the marine algae contains biologically active substances that could be used as antidiabetic agents [7, 13, 15, 16, 30, 36-38].

A synthetic inhibitor can causes side effect such as abdominal pain, diarrhoea and soft faeces in the colon. The present study reveals that the methanol extract of *T. glomerulata* efficiently inhibits the action of α-glucosidase enzymes in the breakdown of starch. The reaction mechanisms involved in inhibition of α-glucosidase enzymes by the methanol extract of *Tolypiocladia glomerulata* need to clearly understood. But there are some suggestions that the presence of chemical constituents such as lignans (quercetin, quercetrin, rutin) and alkaloids in the methanol extracts may be responsible for inhibition activity. However, further *in vivo* studies are needed to confirm the present observations in the *in vitro* studies.

CONCLUSION

The present study concluded that the *in vitro* antidiabetic activity of the methanol extract of red alga *T. glomerulata* is due to the presence of antidiabetic

chemical compounds in the experimental alga. The marine alga showed significant inhibition activity. So the extracts showed close dependent activity upto 87% inhibition against the ezymes. In addition, the extracts are derived from natural resoures could safer to use with reduced side effect. Futher, the importance of the experimentl alga established by further compound isolation, purification and characterization is required to isolate the active enzyme inhibitory component from this alga.

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REFERENCES

1. Kwon, Y. I., Apostolidis, E., & Shetty, K. (2008). *In vitro* studies of egg plant (*Solanum melongena*) phenolics as inhibitors of key enzymes relevant for type 2 diabetes and hypertension. *Bioresource Technology*. 99: 2981–2988.
2. Tiwari, A. K., Srinivas, P. V., Ali, A. Z., Babu, T. H., Nehru, K. J., Agawane, S. B., & Rao, J. M. (2007). Possibility of cost effective management of postprandial hyperglycemic excursion by some common spices. *Indian J. Arecanut. Spices Med. Plants*. 9: 172–178.
3. Shaw, J. E., Sicree, R. A., & Zimmet, P. Z. (2010). Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Research and Clinical Practice*. 87: 4–14.
4. Lebovitz, H. E. (1997). Alpha-glucosidase inhibitors. *Endocrinology and Metabolism Clinics of North America*. 26: 539–551.
5. Santeusanio, F., & Compagnucci, P. (1994). A risk-benefit appraisal of acarbose in the management of non-insulin-dependent diabetes mellitus. *Drug Safety*. 6: 432–444.
6. Kumar, S., Narwal, S., Kumar, V., & Prakash, O. (2011). Alpha glucosidase inhibitors from plants: A natural approach to treat diabetes. *Pharmacognosy Reviews.* 5: 19–29.
7. Lee, S. H., & Jeon, Y. J. (2013). Anti-diabetic effects of brown algae derived phlorotannins, marine polyphenols, through diverse mechanisms. *Fitoterapia*. 86: 129–136.
8. Rindi, F., Soler-Vila, A., & Guiry, M. D. (2012). Taxonomy of marine macroalgae used as sources of bioactive compounds. In M. Hayes (Ed.), Marine bioactive compounds: Sources, characterization and applications. New York: Springer.1–53.
9. Kandra, L., Gyemant, G., Zajacz, A., & Batta, G. (2004). Inhibitory effects of tannin on human salivary α-amylase. *Biochemical and Biophysical Research Communications*, 319: 1265–1271.
10. Apostolidis, E., & Lee, C. M. (2010). *In vitro* potential of *Ascophyllum nodosum* phenolic antioxidant-mediated alpha glucosidase and alpha amylase inhibition. *Journal of Food Science*, 75: H97–H102.
11. Kim, K. Y., Nguyen, T. H., Kurihara, H., & Kim, S. M. (2010). α-Glucosidase inhibitory activity of bromophenol purified from the red alga *Polyopes lancifolia*. *Journal of Food Science.* 75: H145–H150.
12. Moon, H. E., Islam, M. N., Ahn, B. R., Chowdhury, S. S., Sohn, H. S., & Jung, H. A. (2011). Protein tyrosine phosphatase and α-glucosidase inhibitory phlorotannins from edible brown algae, *Ecklonia stolonifera* and *Eisenia bicyclis*. *Bioscience Biotechnology, and Biochemistry:* 75: 1472–1480.
13. Nwosu, F., Morris, J., Lund, V. A., Stewart, D., Ross, H. A., & McDougall, G. J. (2011). Anti-proliferative and potential antidiabetic effects of phenolic-rich extracts from edible marine algae. *Food Chemistry*. 126: 1006–1012.
14. Kawamura-Konishi, Y., Watanabe, N., Saito, M., Nakajima, N., Sakaki, T., & Katayama, T. (2012). Isolation of a new phlorotannin, a potent inhibitor of carbohydrate-hydrolyzing enzymes, from the brown alga *Sargassum patens*. *Journal of Agricultural and Food Chemistry.* 60: 5565–5570.
15. Pandithurai, M., Murugesan, S., Bhuvaneswari, S., & Thennarasan. S. (2015). *In vitro* α-amylase and α-glucosidase inhibition activity of methanolic extract of marine brown alga *Spatoglossum asperum. International Journal of Advances in Pharmaceutics.* 4 (5): 83-87.
16. Murugesan, S., Anand Babu, M., Bhuvaneswari, S., Kotteswari, M., & Thennarasan, S. (2015). *In vitro* antidiabetic activity of methanolic extracts of selected marine algae. *European Journal of Pharmaceutical and Medical Research.* 2 (6): 256-260.
17. Heidari, R., Zareae, S., & Heidarizadeh, M. (2005). Extraction, Purification, and Inhibitory Effect of Alpha-Amylase Inhibitor from Wheat (*Triticum aestivum* Var. Zarrin). *Pakistan Journal of Nutrition* 4 (2): 101-105.
18. Thalapaneni, N. R., Chidambaram, K. A., Ellappan ,T., Sabapati, M. L., & Mandal, S. C. (2008). Inhibition of carbohydrate digestive enzymes by *Talinum portulacifolium* (Forssk) leaf extract. *Journal of Compl. and Integrative Medicine.* 5 (1): 1-10.
19. Tietz, N. W. (1999). In: Burtis, C. A., Ashwood, E. R. Tietz Text book of Clinical Chemistry, third ed. Saunders W.B., pp. 750–778.
20. Matsuura, H., Asakawa, C., Kurimoto, M., & Mizutani, J. **(**2002). α-glucosidase inhibitor from the seeds of balsam pear (*Momordica charantia*) and the fruit bodies of *Grifola frondosa*. *Bioscience Biotechnology and Biochemistry* 66 (7): 1576-1578.
21. Andrade-Cetto, A., Becerra-Jimenez, J., & Cardenas-Vazquez, R. (2008). α-glucosidase-inhibiting activity of some Mexican plants used in

the treatment of type 2 diabetes. *Journal of Ethnopharmacology.*116: 27-32.

22. Lakshmana Senthil, S., Vinoth kumar, T., Geetharamani, D., & Maruthupandi, T. (2013). Screening of seaweeds collected from southeast coastal area of India for α-amylase inhibitory activity, antioxidant activity and biocompatibility. *Int. J. Pharm. Pharm. Sci.* 5 (1): 240-244.
23. Sellappa Sudha., & Palanisamy Senthilkumar. (2012). Evaluation of alpha amylase and alpha glucosidase inhibitory properties of selected seaweeds from Gulf of Mannnar. *Int.Res.Journ. of Pharmacy*. 3 (8): 128-130.
24. Frier, B. M., & Fisher, M. (2006). Diabetes mellitus. In: Boon NA, Colledge NR, Walker BR, Hunter JAA, (Ed.), Davidson's principle and practice of medicine, 20th ed.(Churchill Livingstone Elsevier: Ediburgh 2006) 805-845.
25. Kotowaroo, M. I., Mahomoodally, M. F., Gurib-Fakim, A., & Subratty, A. H. (2006). Traditional medicinal herbs and food plants have the potential to inhibit key carbohydrate hydrolyzing enzymes *in vitro* and reduce postprandial blood glucose peaks *in vivo*. *Phytother. Res.* 20 (3): 228–231.
26. Kostova, I., & Dinchev, D. 2005. Saponins in *Tribulus terrestris* chemistry and bioactivity. *Phytochem. Rev.* 4 (2-3): 111–137.
27. Klein, G., Kim, J., Himmeldirk, K., Cao,Y., & Chen, X. (2007). Evidence-based complementary and alternative medicine. *J. Mol. Biol.* 4 (4): 401–407.
28. Grover, J. K., Yadav, S., & Vats, V. (2002). Medicinal plants of India with anti-diabetic potential. *J. Ethnopharmacol*. 81 (1): 81–100.
29. Louise, L. I., Timothy, J. H., Svend, G., Kaasgaard, C., & Pernille, H. (2006). Structure of *Bacillus halmapalus* α -amylase crystallized with and without the substrate analogue acarbose and maltose. *Protein structure communications*. 62: 849–854.
30. Kim, K.Y., Nam, K. A., Kurihara, H., & Kim, S. M. (2008). Potent α-glucosidase inhibitors purified from the red alga *Grateloupia elliptica. Phytochemistry*. 69: 2820–2825.
31. Kurihara, H., Mitani, T., Kawabata, J., & Takahashi, K. (1999). Inhibitory Potencies of Bromophenols from Rhodomelaceae Algae against. ALPHA.-Glucosidase Activity. *Fisheries science*, *65*(2), 300-303.
32. Davis, S. N., & Granner, D. K. (2001). Insulin, Oral Hypoglycemic Agents and the Pharmacology Endocrine Pancreas. In: Hardman, J. G. and Limbird, L. E., Eds., Goodman and Gilman's T Pharmacological Basis of Therapeutics, McGraw Hill, New York, 1526-1531.
33. Conforti, F., Statti, G., Loizzo, M. R., Sacchetti, G., Poli, F., & Menichini, F. (2005). *In vitro* Antioxidant Effect and Inhibition of. ALPHA.-Amylase of Two Varieties of *Amaranthus caudatus* Seeds *Biological and Pharmaceutical Bulletin.* 28 (6): 1098-1102.
34. Craigie, J. S. (2011). Seaweed extract stimuli in plant science and agriculture. *J. Appl. Phycol.* 23: 371-393.
35. Kurihara, H., Mitani, T., Kawabata, J., & Takahashi, K. 1999. Inhibitory potencies of bromophenols from Rhodomelaceae algae against a-glucosidase activity. *Fish Sci*. 65: 300–303.
36. Xiancui, L., Rough, N., Xiao, F., Lijun, H., & Lixin, Z. (2005). Macroalgae as source of α-glucosidase inhibitors. *Chin. J. Oceanol. Limmol*. 23: 354-356.
37. Benalla, W., Bellahcen, S., & Bnouham, M. (2010). Antidiabetic medicinal plants as a source of alpha glucosidase inhibitors. *Current Diabetes Reviews*. 6 (4): 247-254.