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IN VITRO ANTIDIABETIC ACTIVITY OF METHANOLIC EXTRACTS OF SELECTED MARINE ALGAE

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ABSTRACT

Diabetes mellitus is a chronic disease, which occurs when the pancreas does not produce enough insulin. Marine algae are one of the richest sources of structurally diverse natural products and possess different biological activities. In the present study, three marine algae such as *Ulva lactuca*, *Grateloupia lithophila* and *Stoechospermum marginatum*, were investigated for their antidiabetic potential using *in vitro* enzyme inhibitory assays. Among these *G. lithophila* shows the highest IC_{50} value against α-amylase (IC_{50} 427 µg/mL) and *U. lactuca* shows the highest IC_{50} value against α-glucosidase (IC_{50}760 µg/mL), whereas Acarbose, the positive control shows lesser inhibition against the selected marine algae. This study reveals the possible mechanisms of antidiabetic action *in vitro*. So the further investigation of the antidiabetic activity and identifies the hyperglycemic effect to elucidate their mode of action.

KEYWORDS: *Ulva lactuca*, *Grateloupia lithophila* and *Stoechospermum marginatum*,α-amylase, α-glucosidase, antidiabetic.

1.0 INTRODUCTION

Metabolic disease, including dyslipidemia and diabetes, constitutes a major emerging health crisis in the world.[1] Diabetes mellitus is a chronic disease, which occurs when the pancreas does not produce enough insulin, or when the body cannot efficiently utilize the insulin it produces. This leads to an increased concentration of glucose in the blood. The reason may be lifestyle and genetic factors. [2] In premature atherosclerosis and oxidative stress patient's diabetes is a major risk factor. The World Health Organization, in the 2009 report, states that high blood plasma ranked first in the list of leading global risks for mortality and accounted for 7.5 million deaths in the world in 2004.

The use of natural products or their active components for the prevention and/or treatment of chronic diseases are based on the traditional medicine of various ethnic societies and on epidemiological data [3]. Marine algae are one of the richest sources of structurally diverse natural products. In recent years an increasing number of novel compounds have been isolated from marine algae and many have been reported to possess different biological activities [4-6]. The marine algae have been studied for biologically active components and phlorotannins, marine polyphenols are among them.

Hence, the present study was aimed to evaluate the antidiabetic activity of the selected marine algae such as green algae *Ulva lactuca* (Linnaeus) and brown algae *Stoechospermum marginatum* (C.Agardh) Kützing and red algae *Grateloupia lithophila* Bøergesen.

2.0 MATERIALS AND METHODS

2.1 Preparation of algal material

The marine green algae *Ulva lactuca* (Linnaeus) and brown algae *Stoechospermum marginatum* (C.Agardh) Kützing and red algae *Grateloupia lithophila* Bøergesen were collected from the intertidal regions of Tamilnadu , India, at latitude 9º17' N, longitude 79º 22' E), by hand picking method. The sample was identified by Dr.R.Thevanathan (Retd.), Presidency College, Chennai-5, India. The voucher specimens (PCCACL08, PCCACL09 and PCCACL10) were deposited at the Herbarium Dept of Botany, Pachaiyappa's College, Chennai.

2.2 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 thrice, air dried and stored and then kept in a sealed plastic bag in dry and cool

place to prevent from deterioration. Dried seaweeds were powdered and 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.

2.4 Antidiabetic Activity

2.4.1 Inhibition of alpha 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 alpha amylase in 100 ml of distilled water. The calorimetric reagent was prepared by mixing sodium potassium tartarate solution and 3, 5 di nitro salicylic acid solution (96 mM). The various concentrations of the algal extract (100 to 1000 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 25ºC. Finally the reaction was terminated by adding 1 mL of calorimetric reagent and 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. Control represent 100% enzyme activity and were conducted in a similar way by replacing algal extract with DMSO. A similar experiment was conducted with the standard drug Acarbose and the experiments were conducted in triplicate.

2.4.2 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 extracts for 5 min at 37ºC. The reaction was initiated by adding 1 ml of alpha glucosidase enzyme (IU/ml) to it followed by incubation for 40 min at 37ºC. 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 also for a standard drug acarbose. Percentage of inhibition was calculated by using the following formulae,

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

2.5 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.).

3.0 RESULTS

3.1 Evaluation of *in vitro* α-amylase inhibitory activity

In the present study, the methanolic extracts of marine algae *U.lactuca*, *S. marginatum* and *G. lithophila* were assessed for their inhibition of α-amylase effects on starch break down *in vitro*. The above study showed potent α-amylase inhibitory activity. The crude methanolic extract of *U.lactuca, S. marginatum* and *G. lithophila* at the highest concentration of 1000 µg/mL exhibited α-amylase inhibitory activity of 87.05 ± 0.00%, 89.64 ± 0.00% and 93.25 ± 0.00% respectively (Table.1). The α-amylase inhibitory effects of methanolic extracts of the marine algae were expressed on the basis of their resulting IC_{50} values. There was a dose-dependent increase in percentage inhibitory activity against α-amylase enzyme. *U.lactuca* inhibited the activity of α-amylase with an IC_{50} value of 232 µg/mL, *S. marginatum* with an IC_{50} value of 427 µg/mL and *Grateloupia lithophila* with an IC_{50} value of 318 µg/mL. Acarbose, the positive control used in this study, inhibited the activity of α-amylase with an IC_{50} value of 73 µg/mL (Table.1).

Among the three seaweeds *S. marginatum* showed the maximum inhibition (93.25 ± 0.00%). The methanol extracts of *U. lactuca* showed lesser α-amylase inhibitory activity (87.05 ± 0.00%) comparatively (Table.1).

3.2.2 Inhibition assay for α-glucosidase activity

In general, the activities of marine algal extracts are relatively strong. The methanolic extracts of the selected algae revealed a significant inhibitory action on α-glucosidase enzyme. The crude methanolic extract of *U. lactuca, S. marginatum* and *G.lithophila* at the concentration of 1000 µg/ml exhibited α-glucosidase inhibitory activity of 67.87 ± 0.00, 83.17 ± 0.002 and 87.55 ± 0.06% respectively (Table.2).

Table.1 *In vitro* Alpha-amylase inhibitory activity of marine algae.

S.No	Concentration (µg/mL)	*Ulva lactuca*	*Grateloupia lithophila*	*Stoechospermum marginatum*	Acarbose
1	100	38.29 ± 0.030	14.25 ± 0.002	19.44 ± 0.002	68.54 ± 0.004
2	200	43.13 ± 0.001	18.83 ± 0.030	32.55 ± 0.001	72.12 ± 0.001
3	300	61.33 ± 0.030	20.70 ± 0.001	47.27 ± 0.002	76.19 ± 0.004
4	400	65.85 ± 0.045	30.95 ± 0.001	63.75 ± 0.003	79.23 ± 0.002
5	500	67.04 ± 0.003	58.57 ± 0.030	65.06 ± 0.010	83.82 ± 0.003
6	600	72.11 ± 0.001	65.47 ± 0.003	79.43 ± 0.004	85.99 ± 0.002
7	700	73.14 ± 0.030	75.00 ± 0.00	80.27 ± 0.001	89.22 ± 0.001
8	800	81.57 ± 0.002	77.34 ± 0.002	84.73 ± 0.010	92.13 ± 0.030
9	900	83.04 ± 0.030	85.42 ± 0.002	86.81 ± 0.001	96.34 ± 0.003
10	1000	87.05 ± 0.001	89.64 ± 0.002	93.25 ± 0.002	99.01 ± 0.001
IC_{50} (µg/mL)		232	427	318	73
P – Value		0.000	0.000	0.000	0.000
F – Value		2.342444	2.410966	3.394555	4.648

Table.2 *In vitro* Alpha-glucosidase inhibitory activity of marine algae.

S.No	Concentration (µg/mL)	*Ulva lactuca*	*Grateloupia lithophila*	*Stoechospermum marginatum*	Acarbose
1	100	7.90 ± 0.006	9.97 ± 0.001	16.47 ± 0.007	81.22 ± 0.001
2	200	11.97 ± 0.001	11.87 ± 0.001	18.00 ± 0.000	82.75 ± 0.002
3	300	15.45 ± 0.002	39.77 ± 0.002	31.33 ± 0.055	86.34 ± 0.001
4	400	17.56 ± 0.001	50.77 ± 0.001	43.37 ± 0.077	93.05 ± 0.003
5	500	19.24 ± 0.070	52.87 ± 0.009	49.25 ± 0.067	94.64 ± 0.002
6	600	20.27 ± 0.001	54.97 ± 0.001	51.57 ± 0.000	95.56 ± 0.002
7	700	24.57 ± 0.009	64.57 ± 0.002	55.87 ± 0.008	96.32 ± 0.001
8	800	27.54 ± 0.005	73.57 ± 0.009	66.97 ± 0.067	97.84 ± 0.03
9	900	33.77 ± 0.002	79.17 ± 0.000	73.46 ± 0.028	98.84 ± 0.03
10	1000	65.87 ± 0.002	83.17 ± 0.002	87.55 ± 0.067	99.25 ± 0.03
IC_{50} (µg/mL)		760	394	508	62
P – Value		0.000	0.000	0.000	0.000
F – Value		1.221300	1.554444	3.544444	4.893

The α-glucosidase inhibitory effect of methanolic extracts of the marine algae was expressed on the basis of their resulting IC_{50} values. *Ulva lactuca* inhibited the activity of α-glucosidase with an IC_{50} value of 760 µg/mL, *S. marginatum* with an IC_{50} value of 508 µg/mL and *G. lithophila with* an IC_{50} value of 394 µg/mL and The IC_{50} value of standard drug Acarbose against α-glucosidase was found to be 99.25 µg/mL (Table.2).

Among the three seaweeds *S. marginatum* showed the maximum inhibition (87.55 ± 0.06%) and *U. lactuca* showed lesser α-glucosidase inhibitory activity (65.87 ± 0.00%) (Table.2) comparatively.

4.0 DISCUSSION

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 important as food supplements in order to give good health and resist diseases. 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[7]. A major goal in the treatment of diabetes mellitus is to maintain near normal blood glucose levels in both the fasting and postprandial state [8]. One therapeutic approach to decrease postprandial hyperglycemia is to suppress the production and/or absorption of glucose from the gastrointestinal tract through inhibition of either α-amylase or α-glucosidase enzyme systems decrease the current blood glucose levels in diabetic patient as a short term effect and show a small reduction in glycosylated haemoglobin level as a long term effect.

α-amylase catalyses the hydrolysis of α-1, 4-glucosidic linkages of starch, glycogen and various oligosaccharides and α-glucosidase further breaks down the disaccharides into simpler sugars, readily available for the intestinal absorption. The inhibition of their 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[9]. Therefore, effective and nontoxic inhibitors of α-amylase and α-glucosidase have long been sought. In the human body,

α-amylase is one of the key enzymes that breaks down starch to simpler sugars and increase the absorption rate of glucose. As a consequence, the postprandial blood glucose level is increased.[10-11]

The main effect of diabetes is increasing in glycemic level and therefore to reach normal glycemic level, along with insulin and other oral hypoglycemic agents like sulfonylureas, biguanides [12], Thiazolidinediones (TZD), α-glucosidase inhibitors (AGI) and incretin mimetics (GLP-1, GIP, DPP-4 inhibitors)[13] Garber, 2010 are being used. α-glucosidase inhibitors delay the action of α-glucosidases enzyme to break complex carbohydrates into simple sugars, thereby lowering the absorption of glucose. Acarbose-like drugs inhibit α-glucosidase present in the epithelium of the small intestine has been demonstrated to decrease post-prandial hyperglycaemia[14] and improve impaired glucose metabolism without promoting insulin secretion in NIDMM patients [15].

Therefore, we have screened potential antidiabetic compounds in the algal source for the inhibition of α-glucosidase and α-amylase enzyme activity *in vitro*. The *in vitro* α-amylase inhibitory studies demonstrated that the methanolic extract *U.lactuca, S. marginatum* and *G. lithophila* have an effect α-amylase inhibitory activity. The percentage inhibitory at 100 to 1000 µg/mL concentrations showed a concentration dependant reduction in percentage inhibition. The α-amylase inhibitory activities varied widely among the tested algae. The highest inhibitory activities of these extracts were found to be 93.25 ± 0.00% in *U.lactuca, S. marginatum* and *G. lithophila* (at 1000 µg/mL concentration) and 87.05± 0.02% of inhibitory studies demonstrated that the methanolic extract *U. lactuca* (at 1000 µg/mL concentration), 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[16-17] .

The results of the present study indicate that the methanolic extract of *U.lactuca, S. marginatum* and *G. lithophila* possesses significant *in vitro* antidiabetic activity. The mechanism by which *U.lactuca, S. marginatum* and *G. lithophila* exerted action may be due to its action on carbohydrate binding regions of α-glucosidase enzyme, α-amylase, endoglucanases that catalyze 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. This enzyme is responsible in hydrolyzing dietary starch into maltose which then breaks down to glucose prior to absorption. Since α-amylases play an important role in starch break down in human beings and animals, the presence of such inhibitors in food stuffs may be responsible for impaired starch digestion [18-20].

The inhibition of α-amylase by the extracts of *Ulva lactuca* is a pointer to the fact that the algae is a mild inhibitor of the enzyme, which is desirable in order to prevent some of the side effects produced by synthetic drugs. This is also in agreement with the report of Pinto *et al*. (2009)[21] that the dietary management of hyperglycemia linked to diabetes can be targeted through foods or botanical supplements that have moderate α-amylase inhibition. The characteristic mixed non-competitive inhibition by the methanolic extract of *S. marginatum* and *G.lithophila* suggest that the active components in the extract do not compete with the substrate for the active site of the enzyme, rather the inhibitors bind to a separate site on the enzyme to retard the conversion of substrate to product [22].

The findings of the present study not only clarify the α-amylase inhibitory effect, and the total flavonoid contents of *U.lactuca, S. marginatum* and *G. lithophila*, but also introduce some novel sources for the prevention of non-communicable diseases. It may be due to the presence of chemical constituents such as lignans (quercetin, quercetrin, rutin), and alkaloids in the methanolic extracts. The algae based alpha amylase inhibitor offers a prospective therapeutic approach for the management of diabetes. However, further *in vitro* studies are needed to confirm the present observations.

5.0 CONCLUSION

From the discussion above, it is clear that current pharmacological treatment using synthetic drugs do not adequately control blood glucose levels and their use is hampered by several contraindications and undesirable side effects. Therefore, development of oral agents with a unique mechanism of action is highly desirable. This inhibitory property of the extract may be attributed to the presence of phytochemicals such as saponins and flavonoids. The marine algae showed significant inhibition activity, so further compound isolation, purification and characterization which is responsible for inhibitory activity has been done usage of antidiabetic agent. However, further study is required to isolate the active enzyme inhibitory component from these algae.

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