Research Article

In vitro evaluation of methanolic extract of red seaweeds against α-amylase and α-glucosidase enzyme inhibitory activity

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Abstract

Objective: In the present study, methanolic extract of red seaweeds, *Spyridia filamentosa*, *Grateloupia lithophila* and *Hypnea musciformis* against α- amylase, glucosidase enzyme inhibitory activity were carried out. **Materials and methods:** Phytochemical constituents of methanolic extract were qualitatively determined. Antidiabetic activity was evaluated by inhibitory potential of methanolic extract against α-amylase and α- glucosidase by spectrophotometric assays. **Results:** The crude methanolic extract shows stronger inhibitor effect to α- amylase and α- glucosidase of *S. filamentosa* (IC_{50} = 58.02 and 66.06 µg/ml), *G. lithophila* (IC_{50} = 53.01 and 58.02 µg/ml) and *H. musciformis* (IC_{50} = 48.01 and 51.02 µg/ml), respectively. Phytochemical screening of red seaweed extracts revealed the presence of phenols, protein, lipids and carbohydrates. **Conclusion:** The result suggest that crude methanolic extract of *S. filamentosa* have anti- diabetic potential through inhibition of α- amylase and α- glucosidase.

***Keywords*:** Anti diabetic, Seaweeds, α- amylase, enzymes

Introduction

Hydrolysis of dietary starch is the major source of glucose in the blood, with α-amylase and α-glucosidase being the key enzymes involved in starch breakdown and an intestinal absorption. Enzymes can significantly decrease the postprandial increase of blood glucose level after a mixed carbohydrate diet and therefore can be an important strategy in the management of hyperglycaemia linked to type II diabetes (Kwon et al., 2008). A calcium metalloenzyme, α-amylase catalyses the cleavage of α-D-(1-4) glycosidic linkages of starch, amylose, amylopectin, glycogen and various maltodextrins in to shorter oligosaccharides (De Sales et al.,2012). Consequently, inhibitors of these hydrolytic enzymes suppress the influx of glucose from the intestinal tract to blood vessels resulting in a decrease in postprandial hyperglycaemia.

The uses of seaweeds were 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 (Ribeiro et al.,2000). 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 against several biological activities (Miller et al.,1959). In the present study, to evaluate the phytochemical screening and enzymes activity of red seaweeds (*S. filamentosa*, *G. lithophila*, *H. musciformis*) through *in vitro*.

Materials and methods

Collection of Seaweeds and preparation of extracts

Seaweeds (*S. filamentosa, G. lithophila* and *H. musciformis*) (Figure 1) were collected from Rameshwaram coast of Tamil Nadu, India. Seaweeds specimen were identified by the authentic algal taxonomist. The seaweeds were washed with sea/tap waters and shades dried at 27 °C for within week and were coarsely powdered in a powdering machine. The powdered biomass were prepared using methanol, as solvent using a soxhlet apparatus. The crude extracts were filtered and then concentrated in a rotary evaporator at a temperature 40 ℃ and further used.

Phytochemical analysis of methanloic extract

Preliminary phytochemical screening of methanolic extracts of *S. filamentosa, G. lithophila* and *H. musciformis* were carried out according to standard procedure Harborne (1998).

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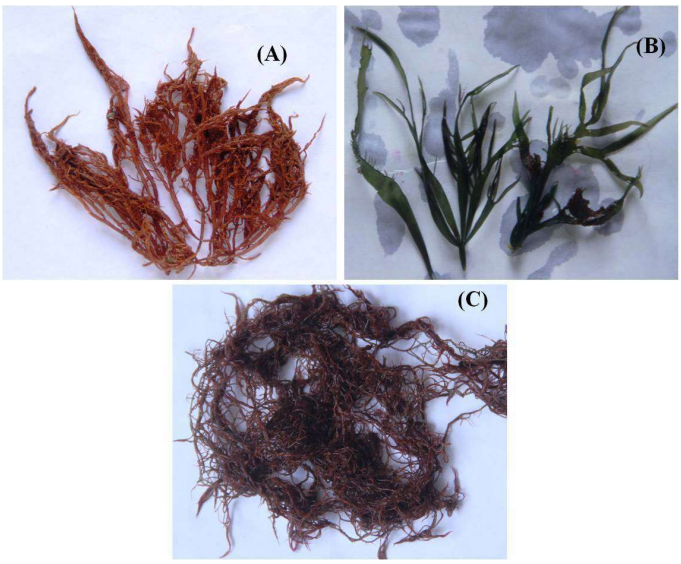

Figure 1. Photographs showing red seaweeds (A) *Spyridia filamentosa,* (B) *Grateloupia lithophila* and (C) *Hypnea musciformis*

α- amylase inhibition assay

The α-amylase was premixed with the methanolic extract at 50 to 250 µg/mL and starch as a substrate was added as a 0.5% starch solution to start the reaction. This was carried out at 37 °C for 5 min and terminated by addition of 2 mL of DNS (3,5-dinitrosalicylic acid) reagent. The reaction mixture was heated for 15 min at 100 °C and diluted with 10 mL of distilled water in an ice bath . α-Amylase activity was determined by measuring spectrum at 540 nm. The IC_{50} value was defined as the concentration of α-amylase inhibitor to inhibit 50% of its activity under the assay conditions (Miller et al., 1959).

α-glucosidase inhibition assay

The inhibition of α-glucosidase activity was determined standard method 3 mM p-nitrophenyl α-D-glucopyranoside as a substrate was added to the reaction mixture. The reaction was incubated at 37 °C for 30 min and stopped by adding 2 mL of Na_2CO_3. The α- glucosidase activity was determined by measuring the p-nitrophenol release from pNPG at 400 nm. The IC_{50} value was defined as the concentration of α-glucosidase inhibitor to inhibit 50% of its activity under the assay conditions (Miller et al., 1959).

$$\% \text{ of inhibition} = \frac{A540 \text{ control} - A540 \text{ Exp.}}{A540 \text{ Control}} X\ 100$$

Data analysis

The α-amylase and α- glucosidase inhibitory activity were performed in triplicate and mean and standard deviation values were calculated.

Results

Phytochemical constituents of the methanolic extracts of *S. filamentosa, G. lithophila* and *H. musciformis* shows in table 1. The most an abundant compounds in *S. filamentosa*, extract were phenols, flavanoids, protein, lipids and carbohydrates (Table 1). Table 1 showed that *G. lithophila* and *H. musciformis* methanolic extracts flavanoids, saponins were not present.

Table 1 Phytochemical constitutes of methanolic extracts of red seaweeds

Constitutes	Methanolic extracts		
	Spyridia filamentosa	*Grateloupia lithophila*	*Hypnea musciformis*
Phenols	+	+	+
Flavanoids	+	–	–
Saponins	+	–	–
Proteins	+	+	+
Lipids	+	+	+
Carbohydrates	+	+	+

α-amylase inhibition activity

The α-amylase inhibition activity of methanolic extracts at various concentrations 50, 100, 150, 200, 250 µg/ml. The inhibition *S, filamentosa* (28%, 33%, 39%, 44% and 58%,), *G. lithophila* (25%, 28%, 33%, 41% and 53%) and *H. musciformis* (22%, 25%, 29%, 32% and 48%) at 50, 100, 150, 200, 250 µg/ml, respectively (Figure2). The standard acarbose 38%, 47%, 59%, 65% and 76% of α-glucosidase inhibition, respectively (Figure 2).

α-glucosidase inhibition activity

Figure 3 showed that α-glucosidase inhibition activity of methanolic extracts at various concentrations 50, 100, 150, 200, 250 µg/ml. The maximum inhibition *S, filamentosa* (66.6%), *G. lithophila* (58.4%) and *H. musciformis* (51.1%) at 250 µg/ml. The minimum inhibition *S, filamentosa* (32%), *G. lithophila* (29%) and *H. musciformis* (25%) at 50 µg/ml, 24hrs (Figure 3). The various range 50 to 250 µg/ml from standard acarbose showed 38% and 76% of α-glucosidase inhibition respectively (Figure 3).

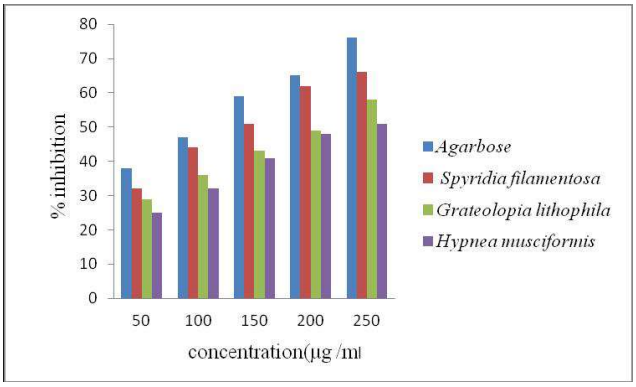

Figure 2. α- amylase inhibition activity of methanolic extracts of selected red seaweeds

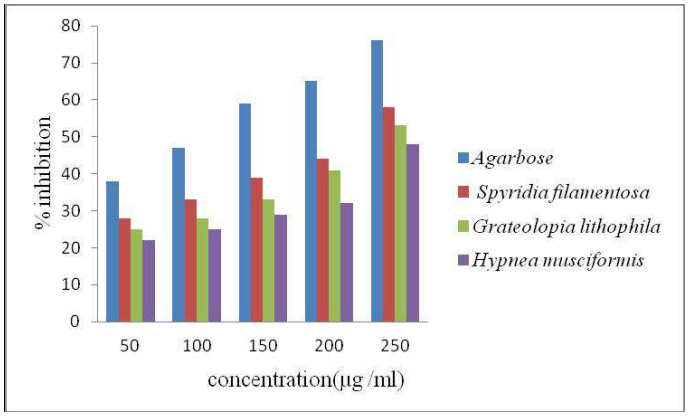

Figure 3. α-glucosidase inhibition activity of methanolic extracts

Discussion

Seaweeds products presents in vitamins, minerals, trace elements, proteins and bioactive substances (Dharmesh et al., 2014). Several polysaccharides are recovered from seaweeds, with the most important of them being agar, alginic acid, laminarine, fucoidin, etc., (Saritha et al., 2013). In the present investigation, phytochemical screening of red seaweeds (*S. filamentosa, G. lithophila* and *H. musciformis*), showed that presence of phenols, flavanoids. proteins, lipids, and carbohydrates, in all extracts were tested. Flavanoids as absent in the methanolic extracts of *G. lithophila* and *H. musciformis.*

Alpha amylase inhibition of seaweeds methanolic extracts of *Chlorodesmis* sp., (green) (Unnikrishnan et al*.,* 2015) and acetone extracts of *Spatoglossum schroederi* (brown) by Teixeira et al (2007). Lakshmana Senthil (2013) studied on α-amylase inhibitory activity using methanol, acetone and ethyl acetate extracts of *Gracilaria edulis* (57±0.9, 53±0.1 and 60±1.2), respectively. Ethanol extracts of *Ascophyllum nodosum* have been strongest a-amylase inhibitory (IC_{50} = 44.7 µg/ml) (Lordan et al., 2013). In the present study, we noted the α-amylase inhibitory activity than acarbose, methanolic extracts of red seaweeds (*S. filamentosa, G. lithophila* and *H. musciformis*).

Sun and Chen (2012) evaluated the inhibitory effects of green algae Chollrella *Pyrenoidasa* sp., against α-amylase and α- glucosidase inhibitory activity and fulfilled an careful as a potential functional food for diabetic patients. The cold water and ethanol extracts of *Fucus vesiculosus* were found to be potent inhibitors of a-glucosidase IC_{50} values of 0.32 and 0.49 µg/ml (Lordan et al., 2013). Reka et al (2017) reported that aqueous and ethanol extracts of *Ulva reticulate*, *Ulva lactuca, Gracilaria edulis*, *Gracilaria corticata* against α-amylase and α-glucosidase at eight hours. The α- glucosidase inhibition activity of *U. lactuca* was 65.71% in ethanol extract.

Conclusion

The methanolic extracts of S. *filamentosa* may be attributed to the presence of phyto-chemicals analysis and inhibitory activity. The marine algae showed significant inhibition activity. Further compound isolation, purification and characterization which is responsible for inhibitory activity has been done usage of anti-diabetic agent.

Acknowledgement

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Conflicts of interest

We declare that we have no conflict of interest.

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