Research Article

Evaluation of *in vitro* Antidiabetic Activity in the Methanolic Extracts of *Protoreaster lincki*

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ABSTRACT

Background: Diabetes is a chronic illness characterized by a metabolic imbalance that is connected to increased and decreased blood sugar levels. Several natural compounds and pharmaceutical drugs have been studied for their antidiabetic effects. **Aim:** The current investigation aimed to assess the methanolic extract of *Protoreaster lincki* in vitro antidiabetic activity using the lipase, α-amylase, and α-glucosidase inhibition assays. **Materials and Methods:** For this investigation, various concentrations of 25 µg/mL, 50 µg/mL, 100 µg/mL, and 250 µg/mL were used. The absorbance values were taken in the spectrophotometer at 580 nm for α-amylase and at 405nm for α-glucosidase and lipase inhibition assay. **Results:** The results show considerable α-amylase, α-glucosidase and lipase inhibition activity in a dose-dependent manner. The lipase assay exhibited more inhibition activity when compared to α-amylase and α-glucosidase assay. Conclusion: This study proves that the methanolic extracts of *Protoreaster lincki* possess antidiabetic activity.

Keywords: *Protoreaster lincki*, Antidiabetic, α-amylase, α-glucosidase, Lipase inhibition.

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INTRODUCTION

Advancing technology and drastic lifestyle changes have been associated with a rise in various non-communicable disorders, including diabetes.[1] If left untreated, hyperlipidemia, oxidative stress, and enzymatic glycation of protein are just a few of the serious complications that can result from diabetes mellitus, a serious complex multifactorial disorder characterized by hyperglycemia and glucose intolerance, either due to a relative deficiency in insulin secretion or impaired effectiveness of insulin action to enhance glucose uptake.[2]

The World Health Organization projects that by 2025, 5.4% of people worldwide will have diabetes, up from 4% in 1995. This increase is mostly expected to occur in developing nations. It is anticipated that the aggregate number of diabetic persons will be around 366 million in 2030.[3] India presently has the largest number of diabetic patients in the world and has been infamously known as the diabetic capital of the world.[4]

As a result of the global prevalence of diabetes, the limited potency and many adverse effects of the medicine presently used, the need for novel diabetes therapies is predicted to expand rapidly within the next decade. Intense research has been performed to uncover new therapeutic targets and pharmacologic agents that might correct poor glucose tolerance. In recent years several research have proven that natural products are a promising source of new treatment candidates for many diseases in general, and diabetes in particular.[5]

The enzymes α-amylase and α-glucosidase play an important role in diabetes. The hydrolysis of starch into a combination of smaller oligosaccharides, including maltose, maltotriose, and oligoglucans, is catalyzed by α-amylase. α-glucosidase breaks these compounds down further to glucose, which is then taken into the bloodstream.[6] Postprandial hyperglycemia is caused by increased activity of α-amylase and α-glucosidase,

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which can damage β-cells, impairing insulin production and reducing glucose uptake.[7,8] The present study has been carried out to evaluate the antidiabetic activity of the methanolic extracts by α -amylase, α-glucosidase and lipase inhibition assay

MATERIALS AND METHODS

Sample Collection

The starfish *Protoreaster lincki* were collected by-catch using fishing nets from the fishing harbor of Thoothukudi coastal area. The samples were thoroughly washed with seawater and cleaned to remove debris. To prevent microbial contamination from the outside, the starfish were carefully packed and stored within plastic bags before being brought to the laboratory. Collected samples are dried under the shade, crushed into small pieces, and powdered.

Preparation of Extracts

The soxhlet device was used to extract the powdered starfish one sample at a time. In 10 millilitres of methanol solvent, 1 g of the powdered starfish sample was extracted. Next, a Whatman No. 1 filter paper was used to filter the extract. The extract was freeze-dried for 48 hr and then promptly frozen at -40ºC. The dehydrated extract was kept at a temperature of -4ºC and then reconstituted in DMSO right before the different bioactivities were measured.

Alpha amylase Inhibition Assay

The alpha-amylase assay was carried out using the procedure that Odeyemi (2015)[9] outlined. 5 L of the enzyme porcine pancreatic solution was put to a 96-well plate together with 15 µl of methanolic extract at varying doses (25 µg/mL-250 µg/mL). The reaction was started by adding 20 µL of starch solution and incubating for an additional 30 min at 37ºC after 10 min of incubation at that temperature. After that, the reaction was halted by adding 75 L of iodine reagent and 10 µL of 1M HCL to each well. A blank was made out of phosphate buffer (pH 6.9) and acarbose (65 µg/mL), the positive control. The absorbance was measured at 580 nm and the percentage of inhibition was calculated by using the following equation:

$$\%\ of\ Inhibition = 1 - \frac{\text{Absorbance of the untreated (control)}}{\text{Absorbance of the test well}} \times 100$$

Alpha-Glucosidase Inhibition Assay

The Alpha glucose inhibition assay was carried out using the Sancheti *et al.*, (2010)[10] method. The alpha-glucosidase solution in the 96-well plate was supplemented with the methanolic extract. Next, 60 µL of potassium phosphate buffer with a concentration of 67 mM (pH 6.8) was introduced. 10 µL of 10 mM p-Nitrophenyl-µ-D-Glucoside (PNP-GLUC) solutions were added after the initial 5-minute incubation period, and the mixture was then incubated for an additional 20 min at 37ºC. Following the incubation period, 25 L of a 100 mM Na2CO3 solution was added, and the absorbance at 405 nm was measured. The positive control utilised in this study was 10 µg/mL of Epigallocatechin Gallate (EGCG). Each sample underwent three separate analyses.

$$\%\ of\ Inhibition = 1 - \frac{\text{Absorbance of the untreated (control)}}{\text{Absorbance of the test well}} \times 100$$

Lipase Inhibition Assay

The procedure Lewis and Liu (2012)[11] outlined was followed in order to determine the lipase inhibition test. The 96-well plates were filled with 10 µL of the methanolic extract at various concentrations (25 µg/mL-250 µg/mL), distilled water as the negative control, and orlistat at a 50 µg/mL as the positive. Following that, a freshly made 10 mg/mL porcine pancreatic solution in 50 mM Tris-HCl buffer (pH 8.0) was centrifuged to remove insoluble material and added to each sample four times its volume. After the initial 15 min of incubation, 18 mL of 50 Mm Tris-HCl buffer (pH 8.0) containing 20 mg gum Arabic, 40 mg sodium deoxycholate, and 100 µL Triton X-100 was mixed with 170 µL of substrate solution (20 mg pNPP in 2 mL isopropanol) and left to incubate for a further 25 min. Absorbance was measured at 405 nm using a spectrophotometer and the percentage of inhibition was calculated using the following equation:

$$\%\ of\ Inhibition = 1 - \frac{\text{Absorbance of the untreated (control)}}{\text{Absorbance of the test well}} \times 100$$

RESULTS

Alpha amylase inhibition assay

In this study methanolic extract of *Protoreaster lincki* was evaluated for its α- amylase inhibition activity against acarbose (positive control). The α-amylase inhibitory activities are presented in Table and Figure 1. The result shows that, the inhibitory activities of methanolic extracts varied from 56.64%-76.94%. As the Concentration of the sample increases the percentage of inhibition also increases. The methanolic extracts of *P. lincki*

exhibited 56.64% inhibition of α- amylase activity at 25 µg/mL, 66.51% at 50 µg/mL, 69.67% at 100 µg/mL and 76.94% at 250 µg/mL concentrations. The positive control acarbose exhibited 92.26%. The IC_{50} value was exhibited at 25 µg/mL.

Alpha glucosidase inhibition assay

The α-glucosidase inhibitory activity of methanolic extracts of *Protoreaster lincki* was observed to be in a concentration-dependent manner. The percentage of inhibition ranged from (43.45%-83.56%). The results of α glucosidase inhibition assay are summarized in Table 1 and Figure 2. The methanolic extract at 25 µg/mL, 50 µg/mL, 100 µg/mL and 250 µg/mL shows inhibition activity of 43.45%, 47.41%, 59.31% and 83.56% respectively. Against positive control EGCG (63.64%). The IC_{50} value was recorded at 100 µg/mL of sample.

Lipase Inhibition assay

The extracts showed antidiabetic lipase inhibition assay as shown in Table 1 and Figure 3. The lipase inhibition activity of the methanolic extract at different concentrations (25 µg/mL, 50 µg/mL, 100 µg/mL, 250 µg/mL) was recorded. *P. lincki* showed maximum inhibition 85.67% at 250 µg/mL and the minimum inhibition of 55.20% at 25 µg/mL. An inhibition of 68.79% at 50 µg/mL and 75.16% at 100 µg/mL was also observed. The positive control orlistat exhibited 62.42% of inhibitory activity at 50 µg/mL. The exhibit IC_{50} value was observed at 25 µg/mL.

Table 1: *In vitro* antidiabetic activity of methanolic extract from *Protoreaster linckii*

Concentration (µg/mL)	% of Inhibition		
	α-amylase	α-glucosidase	Lipase Inhibition
250	76.94	83.56	85.67
100	69.67	59.31	75.16
50	66.64	47.41	68.79
25	56.64	43.45	55.20

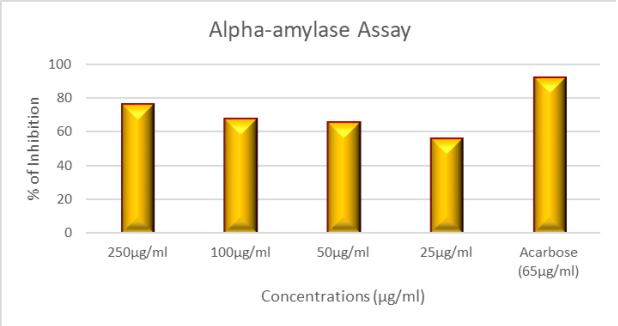

Figure 1: *In vitro* Antidiabetic activity of α-amylase assay

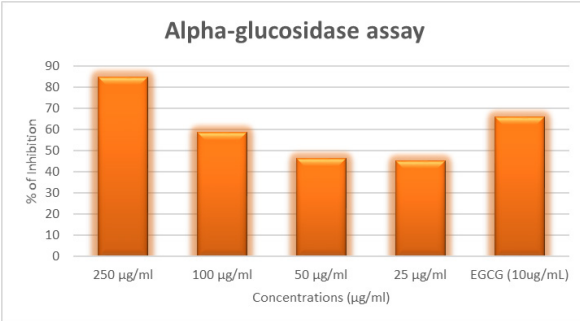

Figure 2: *In vitro* Antidiabetic activity of α-Glucosidase assay

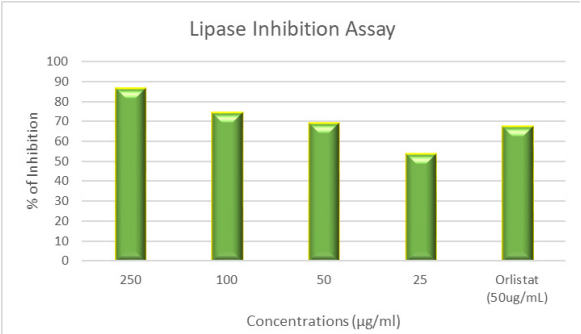

Figure 3: *In vitro* Antidiabetic activity of Lipase Inhibition assay

DISCUSSION

Diabetic is a chronic disorder, a fatal disease that is increasing day by day[12] (Romila *et al.*, 2010). Worldwide, the fast rise of diabetic poses a major risk to people's health (Neelesh Malviya *et al.*, 2010).[13]

The current study's results unequivocally showed that *P. linckii* methanolic extract has antidiabetic potential. *In vitro* antidiabetic activities were observed through α- amylase, α- glucosidase and lipase inhibition assay. α-amylase assay showed an antidiabetic activity of 76.94% inhibition at 250 µg/mL, and at a concentration of 25 µg/mL, it showed 56.64% inhibition. Similarly Marmouzi *et al.*, 2018[14] carried out antidiabetic activity in different species of starfish such as *Asteropecten irregularis*, *Luida Sarsi* and *Ophiura albida*. In α-amylase activity, the IC_{50} values for *A. irregularis*, *L. Sarsi* and *O. albida* extracts shows 147.08 µg/mL, 150.52 µg/mL and 737.32 µg/mL respectively, indicating their high effectiveness.

α-glucosidase assay in the methanolic extract exhibited highest inhibitory activity with the percentage of inhibition 83.56% at 250 µg/mL and 43.45% at 25 µg/mL compared to the positive control EGCG (63.64%). In corroborate with the present study Marmouzi *et al.*, 2018 observed α-glucosidase activities in the starfish *Asteropecten irregularis*, *Luida Sarsi* and *Ophiura albida* against acarbose as positive control. The three tested

starfish extracts displayed inhibitory activities ranging from 442.76 µg/mL-872.29 µg/mL.

Mohamed *et al*., (2021)[15] isolated the compounds from the large starfish *Acanthaster planci*. He showed that the compounds, 5α-cholesta-24-en-3β, 20β-diol-23-one and 5α-cholesta-9(11)-en-3β, 20β-diol which are responsible for the antidiabetic activity. These compounds exhibited α-glucosidase activity with respective IC_{50} values of 58±0.8 and 55±0.5 µg/mL, while acarbose, used as a positive control had an IC_{50} of 36±0.4 µg/mL. This is in agreement with the present study.

The lipase inhibition assay is commonly used to evaluate the antidiabetic activity of compounds by measuring their ability to inhibit enzyme lipase, which is involved in the breakdown of dietary fats. In the present study, the extract exhibited excellent lipase inhibitory activities in the range of 55.20% to 85.67% while the positive control orlistat was 52.42%. As the concentration of methanolic extracts increased the inhibition rate also increased. Among all the three assays, lipase inhibition assay showed better antidiabetic activity when compared to α- amylase and α-glucosidase assays. The above results suggested that the methanolic extracts of *Protoreaster linckii* show significant antidiabetic properties in in vitro models

CONCLUSION

The methanolic extract of *Protoreaster linckii* exhibited significant antidiabetic activity in the alpha-amylase, alpha-glucosidase and lipase inhibition assay. Therefore, additional compound isolation, purification, and characterization that accounts for the inhibitory action must be assessed to rule out the possibility of using it as an antidiabetic treatment in the future.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

µg: Microgram; **mL:** Millilitre; **Mm:** Millimetre; **α:** Alpha; **NO:** Number; **DMSO:** Dimethyl Sulfoxide; **C:** Celsius; **HCl:** Hydrogen chloride; **pH:** Potential of hydrogen; **Na_2Co_3:** Sodium carbonate; **EGCG:** Epigallocatechin gallate; **IC_{50}:** Half maximal inhibitory concentration.

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