



**MOLECULAR CHARACTERIZATION OF PROTEIN AND ANTIDIABETIC
EFFICACY OF THE MARINE GASTROPOD *LAMBIS LAMBIS***

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ABSTRACT

Diabetes mellitus is a major global health challenge of current century afflicting over 366 million people world-wide and by the year 2030, this endocrine disorder is predicted to affect over 552 million people, particularly from the middle or low wage countries. The main objective of this study is to characterize the molecular weight of protein and the antidiabetic potential of marine molluscs *Lambis lambis*. The protein was isolated using thin layer chromatography, SDS-PAGE and *in vitro* antidiabetic activity using inhibition of alpha amylase and alpha glucosidase activity. The R_f value was calculated and it corresponds to five different amino acids respectively. The clear bands detected in the SDS-PAGE gel indicate the molecular weight of 60 kDa. The percentage inhibition found to be increased with increase in the concentration against α -amylase and α -glucosidase enzymes.

**Keywords: Gastropod, *Lambis lambis*, Methanolic extract, TLC, SDS-PAGE,
Antidiabetic activity**

INTRODUCTION

Within, marine environment ecosystem, from estuaries to the deep invertebrates represent a great majority sea [1]. Marine organisms are used as of all macroscopic life and inhabit all nutritious foods, animal feed, ornamental

and recreational items and also as potential source of marine natural products in health care since ancient times. Molluscs which are widely distributed throughout the world, have many representatives in the marine and estuarine ecosystem.

Synthesis of secondary metabolites is their unique ability that helps them to protect against the ill effects of environmental hazards and microbial infections and many of these are found as promising source to meet the human health care demand [2]. Molluscs are considered as one of the important sources to derive bioactive compounds that exhibit antitumor, antimicrobial, anti-inflammatory and antioxidant activities [3-5].

Marine molluscs have become the focus of many chemical studies aimed at isolating and identifying novel natural products. The secondary metabolites isolated from molluscs fall into a wide range of structural classes, with some compounds predominating in certain taxa. Usually for bioprospecting, freeze - dried samples of marine organisms are solvent extracted and the extract is partitioned by various chromatographic techniques including thin layer chromatography, vacuum liquid chromatography, column chromatography

and high - performance reversed - phase liquid chromatography [6].

A preliminary thin layer chromatography was used to determine the qualitative status of free amino acids [7]. The protein contained nutritionally useful quantities of most of the essential amino acids, including sulphur containing amino acids [8]. SDS-PAGE is the most widely used method for analyzing protein mixture qualitatively. It is particularly useful for monitoring protein purification and this method is based on separation of proteins according to their size. It can also be used to determine the relative molecular mass of proteins [9].

Screening of organic extracts from marine organisms is a common approach to identify compounds of biomedical importance. *Diabetes mellitus* is ranked seventh among the leading causes of death and is considered third when its fatal complications are taken into account. For people with diabetes, either not enough insulin is produced or it is not working properly and therefore, glucose builds up in the blood [10]. Statistical projections about India suggest that Indians are genetically more susceptible to diabetes, and the World Health Organization predicts the number of diabetic person in India would go up to

40 million by 2010 and to 74 million by 2025 [11].

The deficiency of insulin leads to *Diabetes mellitus* (DM). The World Health Organization reported that 5.0 % of global population has been affected by *Diabetes mellitus* and at present, there is no drug to give permanent remedy to *Diabetes mellitus* [12]. Alpha amylase and glucosidase are the key hydrolysing enzymes involved in the digestion of carbohydrates [13]. Inhibition of these enzymes resulted in decreasing the rate of digestion of carbohydrate, prolonged the time of the carbohydrate digestion and thereby reduced the amount of glucose level in blood. The antidiabetic agent which was isolated from molluscs and studied in rat models showed significant hypoglycemic effect [14]. So, the present study has been carried out to investigate the biomedical efficacies such as protein isolation through TLC, SDS – PAGE and antidiabetic activity of the marine gastropod *Lambis lambis*.

MATERIALS AND METHODS

Collection of experimental organism

In the present study the mesogastropod *Lambis lambis* was collected from the Thoothukudi coastal region. The mesogastropod *L.lambis* was

collected from the landed by-catch from fishing trawlers operated for crabs and prawns along the Therespuram, Thoothukudi coastal region. The freshly collected samples were brought to the laboratory, cleaned and washed with fresh sea water to remove all impurities. The shells were broken, tissues were removed and then dried in hot air oven at 56°C for 48 hours and used for further studies.

Thin layer chromatography (TLC) analysis

Thin layer plates of silica gel were used for qualitative (chromatographic) analysis of secondary metabolites (i.e for separation of proteins, peptides and amino acids). Thin plates of uniform thickness were prepared using silica gel. The methanolic extract of gastropod were loaded on TLC plate with the help of micropipette for the separation of peptides and amino acids. The solvent employed was butanol, acetic acid and distilled water in the proportion of 5:1:4. When the TLC plate was sufficiently developed it was removed from the glass jar, air dried and sprayed with Ninhydrin reagent (0.5% solution of Ninhydrin in 100 ml 80% acetone). The pink spots were developed which indicated the presence of proteins, peptides and amino acids.

$$R_f = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

Molecular characterization of protein (SDS - PAGE)

SDS-PAGE is the most widely used method for analyzing protein mixture qualitatively. SDS - PAGE was performed following the method described by Laemmli [15].

In vitro antidiabetic activity

Inhibition of alpha amylase activity

The alpha amylase activity was done according to the method of Thalapaneni *et al* [16] and Heidari *et al* [17]. A total of 0.5 ml of different concentrations of sample i.e. 100 µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml, 500

µg/ml were taken from test samples. Control represent 100% enzyme activity and were conducted in similar way without adding the sample extract.

Inhibition of alpha glucosidase activity

The inhibitory activity was measured by glucose oxidase peroxidase method of Adolfo Andrade-Cetto *et al* [18]. The concentration of the gastropod extracts required to scavenge 50% of the radicals (IC₅₀) was calculated by using the percentage scavenging activities at five different concentrations of the extract. Percentage inhibition (I %) was calculated by:

$$\text{Percentage Inhibition} = \frac{\text{Absorbance of Control} - \text{Absorbance of Test}}{\text{Absorbance of Control}} \times 100$$

RESULTS

Thin layer chromatography

TLC profiling was done for the methanolic extract of *Lambis lambis* in solvent system of n- butanol: acetic acid: water (B:A:W) in proportions of 6:2:2. The plates were developed in the solvent systems and showed the development of pinkish colored spot in tissue extract, when the TLC plate was sprayed with ninhydrin. The plate with fractions when developed in BAW as

the solvent system and sprayed with ninhydrin, showed pink spots indicating the presence of amino acids and peptides. The R_f value was calculated as 0.20, 0.29, 0.39, 0.42 and 0.73. The R_f value corresponds to five different amino acids visualizing arginine, histidine, threonine, cystine, phenyl alanine (**Figure 1**).

SDS – PAGE

The test sample was subjected to SDS - PAGE to estimate the molecular

weight of active proteins present in it. The stained gel revealed that the sample contained a simple population of proteins. The clear bands detected in the gel represent the molecular weight of 60 kDa (**Figure 2**).

Antidiabetic activity

In vitro α -amylase activity

The methanol extract of *L. lambis* revealed a significant inhibitory action on α -amylase enzyme. The percentage inhibition at 100 – 500 $\mu\text{g/ml}$ concentrations of *L.lambis* extract showed a concentration dependent increase in percentage inhibition. The percentage inhibition varied from 26.03% to 76.71% for various concentrations of 100 to 500 $\mu\text{g/ml}$.

The percentage inhibition was found to be maximum of 76.71% at 500 $\mu\text{g/ml}$ of concentration followed by 62.32% at 400, 54.11% at 300, 34.93% at 200 and minimum of 26.03% at 100 $\mu\text{g/ml}$ of concentration respectively. The 50% inhibition (IC_{50}) was found to be 280 $\mu\text{g/ml}$ respectively (**Figure 3**).

In vitro α - glucosidase activity

There was a dose - dependent increase in percentage inhibitory activity against α -glucosidase enzyme. *L. lambis* showed 32.78% at 100 $\mu\text{g/ml}$ followed by 39.67% at 200 $\mu\text{g/ml}$, 58.08% at 300 $\mu\text{g/ml}$, 65.02 % at 400 $\mu\text{g/ml}$ and 86.71% at 500 $\mu\text{g/ml}$ concentration respectively. The IC_{50} value was found to be 251.58 $\mu\text{g/ml}$ (**Figure 4**).



Figure 1: Thin layer chromatography of *Lambis lambis*

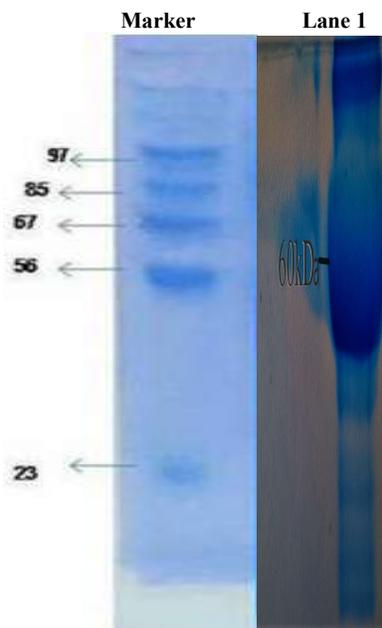


Figure 2: Molecular weight determination of protein using SDS – PAGE

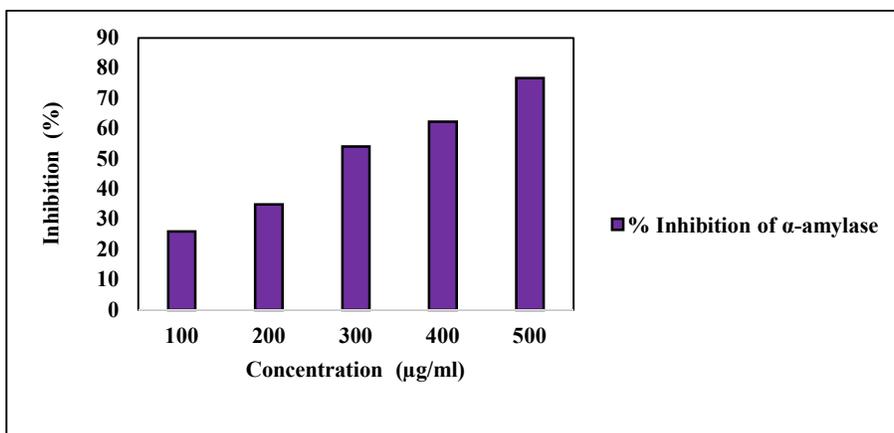


Figure 3: Antidiabetic activity of *Lambis lambis* by α - amylase activity

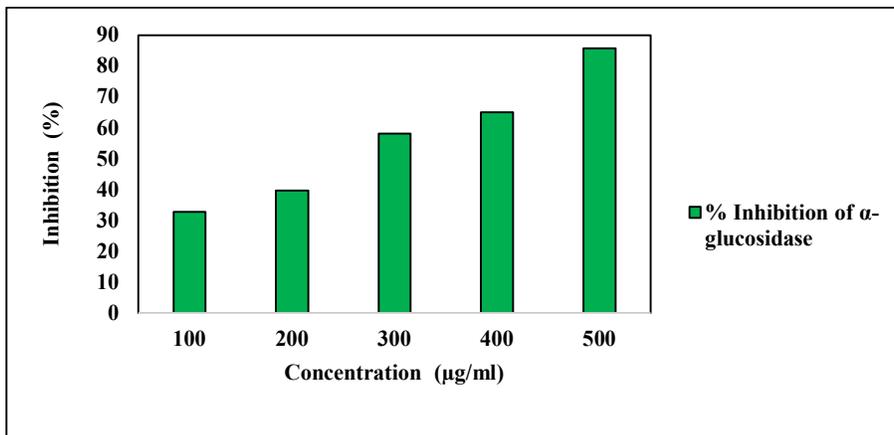


Figure 4: Antidiabetic activity of *Lambis lambis* by α - glucosidase activity

DISCUSSION

Marine organisms were shown to be rich sources of bioactive compounds, which have a positive influence on human health and may open a new perspective for pharmacological development [19-22]. The present study is the first of its kind to report the biomedical properties of the marine gastropod *L. lambis* with regard to its protein isolation and antidiabetic activity by various *in vitro* mechanisms.

Protein is a major biochemical constituent in all invertebrate and received high attention due to their potential bioactive and functional properties [23]. Dolastatins are a group of cyclic and linear peptides isolated from the marine mollusc *Dolabella auricularia*, with prominent cell growth suppressing activity.

Bragadeeswaran *et al* [24] studied ascidian extracts of TLC which showed the R_f value of 0.2 and 0.3 with ethyl acetate: dichloromethane solvents (9:1 v/v), that the results alkaloids and peptides were clearly detected. Arularasan *et al* [25] studied thin layer chromatography with solvents mixture of n - Butanol: Acetic acid :Water (6:2:2 v/v) clearly showed the R_f value of 0.92 and 0.87 respectively. In the present study, thin layer chromatography was carried out in the methanolic extract of

Lambis lambis. The result revealed that R_f value of 0.20, 0.29, 0.39, 0.42 and 0.73 corresponds to five different amino acids (Figure 1).

Sugesh and Mayavu [26] reported SDS-PAGE on 12% gel, the crude proteins of *M.meretrix* and *M.casta* showed 5-6 bands ranged from 45-223 kDa. Sumita *et al* [27] observed that unclear bands ranging from 14 and 29 kDa in marine bivalves *M.casta* and *P.viridis*. Scotti *et al* [28] observed the similar result from *Perna canaliculus* containing the protein with molecular weight of 35 kDa. Chandran *et al* [29] observed 9.7 kDa proteins in estuarine bivalve *P.viridis*. Very similar to the present study a 60 kDa protein was isolated from the secreted purple fluid of *A.kurodai* and called *Aplysianin P*. *Aplysianin P* displayed cytolytic and antibacterial effects. The present study suggests that methanolic extract of *L.lambis* showed the presence of 60 kDa clear band (Figure 2).

Anti α - amylase activities of the molluscs were supported by Sadhasivam *et al* [30] explained the α -amylase inhibitory properties of the methanolic extract of three marine molluscs namely *Aplysia sp*, *Bursatella leachii* and *Kalinga ornata* (93.0, 70.6 and 50.0% respectively at 0.1mg/ml). Abirami *et al* [31] also observed moderate α - amylase

inhibitory activity by the purple fluid of the marine gastropod *Dolabella auricularia*. An α - amylase inhibition of 72% was observed by Ravi *et al* [32] for the methanolic extract of two marine molluscs *Hemifusus pugilinus* and *Natica didyma*. He also observed that the methanol extract of gastropod *Hemifusus pugilinus* exhibited greater anti - α - glucosidase activity (IC_{50} 20.27 mg/ml) than the methanol extract of *Natica didyma* (IC_{50} 56.44 mg/ml), although the anti - diabetic properties of this group of molluscs were significantly lesser than the EtOAc-MeOH extracts of the cephalopods *A.marginatus*, *U.duvauceli*, *S.pharaonis*, *S.inermis* and *C. indicus* (IC_{90} 1.69- 5.37 mg/ml; $p < 0.05$). The methanol extract of the marine gastropod *Cerithidea obtusa* extract was found to possess moderate anti- α -glucosidase inhibitory activity (IC_{50} 36.40 mg/ml) [33]. In the present study, IC_{50} value of α - glucosidase activity was found to be 251.58 μ g/ml.

The α - amylase inhibitory activities of *C.indicus*, *S.inermis* and *U.duvauceli* were recorded to be significantly greater (IC_{90} ~ 1.7 mg/ml) when compared with *A.marginatus* and *S.pharaonis* (IC_{90} 1.9-2.5 mg/ml; $p < 0.05$). *A.marginatus* displayed least α -amylase inhibitory activity (IC_{90} 2.5mg/ml). Therefore, the antidiabetic

potential of EtOAc-MeOH extract of cephalopods by α -amylase inhibition demonstrated its effectiveness as an anti - diabetic agent. The solvent extracts derived from the members of the order Octopoda demonstrated fairly good α -amylase inhibitory activity ($IC_{90} \leq 2.5$ mg/ml) and in which *C.indicus* displayed highest anti- α -amylase property (IC_{90} 1.69 mg/ml). In the present study, the 50% inhibition of α - amylase was found to be 280 μ g/ml respectively. Thus, the use of glucosidase inhibitor, such as α -amylase and α -glucosidase inhibitors, would be a prospective therapeutic agent for the effective management of diabetes [34]. In the present study the methanol extract of *L.lambis* showed good α - amylase inhibitory activity. The present study unequivocally proved that experimental organism *L.lambis* belonging to Mesogastropoda possess potential bioactivities capable of inhibiting the α -amylase and α -glucosidase enzymes. Therefore, this species can be considered to be the potential candidate species for use against *Diabetes mellitus*.

CONCLUSION

The present study concluded that the *Lambis lambis* extract showed good biomedical potential. The results suggest that methanol extract of *L.lambis*

efficiently inhibits α -amylase and glucosidase enzymes *in vitro*. The percentage inhibition at 100-500 $\mu\text{g/ml}$ concentrations of methanolic extract of *Lambis lambis* showed a concentration dependent increase in percentage inhibition. The antidiabetic action of *L.lambis* can also be attributed to the intestinal α -glucosidase inhibitory activity. The recent research findings have further shown that many more species of marine organisms, particularly the molluscs, stand as a prospective source of valuable bioactive compounds with great potential for new leads.

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