International Journal of Pharmacy and Biological Sciences-IJPBS™ (2019) 9 (3): 12-15
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Biological Sciences | Open Access | MCI Approved
UGC Approved Journal

Evaluation of *In Vitro* Antidiabetic Effect of Chitosan from Lobster (*Panulirus ornatus*) Shell

Balu Arasukumar[a], Gopal Prabakaran[a] and B. Gunalan[a,b*]
[a]Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai 608 502, Tamil Nadu, India.
[b]PG and Research Department of Zoology, Thirukilanchiappar Government College of Arts College, Virudhachalam - 606001, Cuddalore (Dist), Tamil Nadu, India.

Received: 12 Mar 2019 / Accepted: 19 Apr 2019 / Published online: 1 Jul 2019
Corresponding Author Email: aquagunal@gmail.com

Abstract
The present study was aimed to extract the chitosan from the chitin complex by chemical method and screening their antidiabetic potential by *in vitro* assays from the lobster *Panulirus ornatus* shell. The chitin yield was calculated for 21% and the chitosan was obtained from the chitin by deacetylation process and the yield was found to be 35%. The α-amylase inhibition potential of chitosan has showed concentration depended and the maximum activity 64% was recorded at 1000 µg/ml and the minimum activity 16% was recorded at 200µg/ml. The β-glucosidase inhibition potential of chitosan has showed dose depended and the maximum activity was 43% showed at 1000µg/ml and the minimum effect 11% was noticed 200µg/ml. Chitosan and alternate source for the development of antidiabetic drug development.

Keywords
Chitin, Chitosan, deacetylation, α-amylase, β-glucosidase

*****

INTRODUCTION
The crustaceans are one of the abundance marine organisms and have particular biological characteristics features; particularly having exoskeleton made up of the polysaccharide chitin and calcium. The outer shell, in addition to being protective, gives rigid support for the attachment of the muscles. While crustaceans grow, their outer shell, the exoskeleton, does not grow with them, so they must regularly shed these shells in order to growth in size. This process is well known as molting; it occurs periodically when the body is ready to increase in height and weight and involves the detachment of the exoskeleton

Chitosan is a natural linear polyelectrolyte at acidic pH; it has a high charge density, one charge per each glucosamine unit. The chain structure has positively charged amine functional groups which are responsible for the polyelectrolyte behavior. Chitosan can coagulate negatively charged material with its positively charged functional groups to give electric neutrality (Rinaudo, 2006).

Chitosan is characterized by its biocompatibility, biodegradability and non-toxicity, its exceptional biological properties (antimicrobial, antibacterial and coagulating activities, bio adhesivity and wound healing capacity) have prepared it an excellent candidate for applications in cosmetics, medicine and pharmacy, agriculture industries. The chitosan in the preservation of agricultural commodities, the food industry and wastewater treatment engineering among several other industrial applications (Dash et

al., 2011; Khor and Wan, 2013; Bouhenna et al., 2015). Deacetylation of chitin to produce chitosan is usually attained by hydrolysis of the acetamide groups by intense NaOH or KOH (40–50%) at temperatures above 100°C. This reaction is generally carried out under varied conditions. The acetylation degree (DA) of chitosan, defined as the proportion of acetylglucosamine units in the polymer, will based on the deacetylation reactions. The present study was undertaken to extract the chitosan from the chitin and screening their antidiabetic potential by *in vitro* methods.

MATERIALS AND METHODS

Collection of shells and processing

The *Panulirus ornatus* lobster was collected from the Nagapattinam fish landing center (Nagapattinam Dist, Tamil Nadu; 10.7656° N, 79.8424° E) and the lobster species was identified by using the morphological key characters and shells were dissected out, washed with distilled water and air dried in room temperature.

Chitin extraction

The air-dried shells taken and pulverized by mortar and pestle, the shell powder was used for chitin extraction process. The chitin extraction was performed by following the method of Takiguchi (1991). Chitin was extracted from the lobster shell powder through demineralization and deproteinization process, the powder was treated with 2N HCl and warmed for 24 h to eliminate the minerals and early reacts with 1N NaOH at 80°C and allow to standing for 24 h for the removal of protein molecules.

Preparation of chitosan

The chitosan was obtained from the chitin through deacetylation process by the method of Takiguchi (1991). The deproteinized chitin was deacetylated with 250 ml of 40% NaOH and heat for 6 h at 110◦C in a continuous stirring. The suspension was filtered and then the filtrate was washed previously with 200 ml of 10% acetic acid was added to the sample and kept for 12 h at room temperature in magnetic stirrer. The dissolved sample matrix was reprecipitated by addition of 40% NaOH and adjusts the pH to 10. After that the sample was dialyzed by deionized water to a pH of 6.5, then centrifuged at 10,000 rpm for 10 min and the precipitate was lyophilized for further analysis.

Screening of α-amylase inhibition effect

The α-amylase enzyme inhibition effect of the chitosan was screened by Apostolidis and Lee (2010). The 100µl of chitosan was taken 200, 400, 600, 800 and 1000 µg/ml for this assay and the same concentrations of Standard (Acrbose) was taken for standard, the porcine pancreatic amylase (Sigma) was used and the sample and standard was take at absorbance of 540 nm by spectrophometer (Shimadzu). The blank tubes were prepared by replacing the enzyme solution with 200 µL in distilled water.

Screening of β-glucosidase inhibition effect

The *in vitro* β-glucosidase inhibitory potential of was carried out by following the method of Kim *et al*. (2011). 1 mg of glucosidase enzyme (Sigma) was dissolved in 100 ml of phosphate buffer (pH 6.8). The 100µl of sample at various concentrations such as 200, 400, 600, 800 and 1000 µg/ml was taken and added 200µl of glucosidase enzyme then the mixture was incubated at 37ºC for 20 min and the same concentrations of Acrbose (Standard) was taken for comparison. The β-glucosidase activity was determined spectrophotometry at 405 nm on spectrophotometer UV-VIS (Shimadzu UV-1800) by measuring the quantity of nitrophenol released from p-NPG.

RESULTS AND DISCUSSION

Chitosan, have been achieved through partial or total deacetylation of chitin and it is one of the most presented polysaccharides in nature, and a well-known and high valuable bio material for the production of packaging of some materials because of the attractive combination of price, abundance and thermal withstand plastic characters, apart from its more hydrophobic nature as compared to starch and additionally, chitosan is non-toxic, biodegradable and has antimicrobial activity (Matet *et al.,* 2014). In the present the chitin was extracted by deproteinization and demineralization process and the yield was calculated as 21% and chitosan was found to be 35% respectively, which is lower yield than the previous study of Anderson et al. (1998) who have been reported the chitin from prawn shells and shellfish waste materials and noticed the highest chitin yield (80% and 27%) respectively. Synowiecki and Al-Khateeb (1997) extracted chitosan from the mycelia of *Mucor rouxii* and examined the influence of growth time on the contents of chitosan and other main components of *M. rouxii* mycelia, as well as the yield of chitin and chitosan during the isolation process. The antidiabetic activity of the chitosan was assessed by *in vitro* assay namely, α-amylase and β-glucosidase enzyme inhibition method and the results were shown in Fig.1 and Fig.2. The α-amylase inhibition potential of chitosan has showed concentration depended and the results has showed 16, 22, 35, 49 and 64% at the concentration of 200-

1000µg/ml and the standard acarbose 31, 47, 58, 81 and 95% at the concentration of 200-1000µg/ml respectively, the maximum enzyme activity was recorded in high concentration of chitosan and acarbose. The β-glucosidase inhibition potential of chitosan has showed dose depended and the results has showed 11, 17, 31 and 43% at the concentration of 200-1000µg/ml and the standard acarbose 27, 41, 63, 81 and 94 % at the concentration of 200-1000µg/ml respectively and the maximum enzyme activity was recorded in high concentration of chitosan and acarbose. Similarly, Zhang (2014) investigated the β-glucosidase inhibition effect of chitosan, catechin-g-chitosan, catechin and acarbose and noticed the effect for 27.06, 72.45, 58.86 and 36.65% at the concentration of 1 mg/ml and the α-amylase inhibitory effects of chitosan and catechin-g-chitosan are 17.65, 36.47, 32.35 and 62.94% at the concentration of 10mg/ml respectively. Correspondingly, Tarafdar and Biswas (2013) extracted the chitosan from prawn shell and reported the several potential biomedical applications *in vitro* level. The chitosan of the lobster *Panulirus ornatus* has showed potential antidiabetic *in vitro* level.

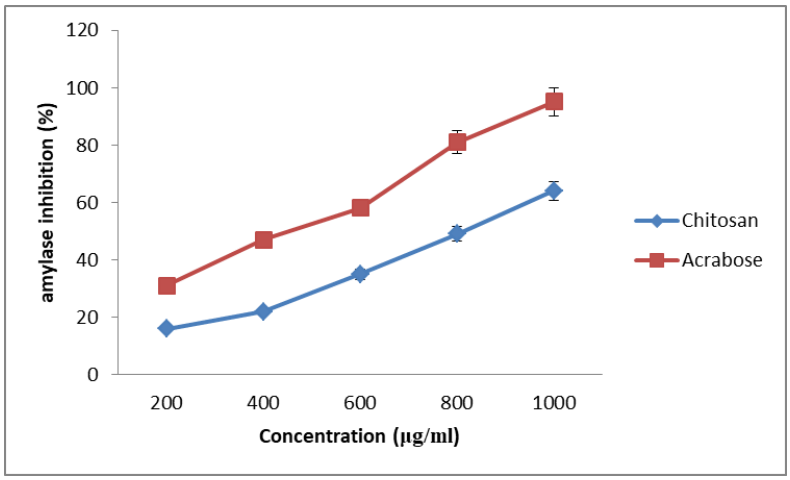

Fig.1. α-amylase inhibition effect of chitosan from *Panulirus ornatus*

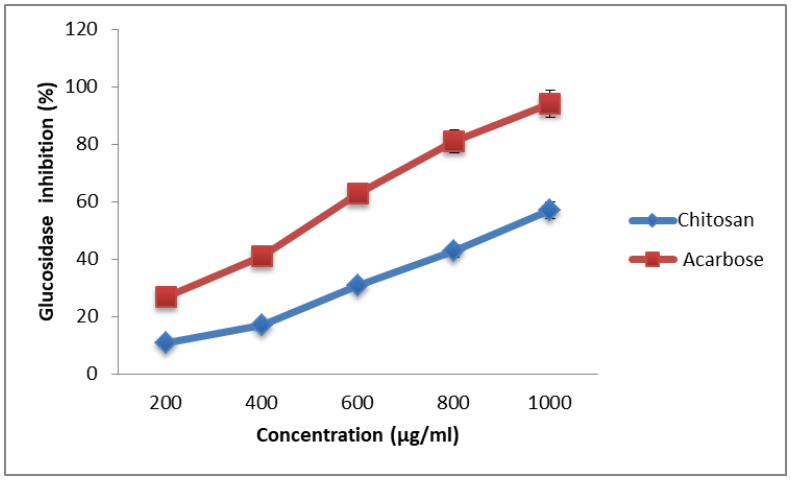

Fig.2. β-glucosidase inhibition effect of chitosan from *Panulirus ornatus*

CONCLUSION

The present study results have showed *Panulirus ornatus* shells comprised more chitin complex than the other marine organisms except crustaceans and the shell derived chitosan polymers are have potential α-amylase and β-glucosidase inhibition effect. In conclusion, the chitosan is a potential and alternate source for the development of antidiabetic drug development in pharmaceutical industry.

Acknowledgement

Authors are thankful to the Dean and Director, CAS in Marine Biology, Faculty of Marine Sciences, Annamalai University for providing all necessary facilities.

REFERENCES

Apostolidis, E., Lee, C. (2010). In vitro potential of Ascophyllum nodosum phenolic atioxidant-mediated α-glucosidase and α-amylase inhibition. *Journal of Food Science, 75,* H97–H102.

Bouhenna, M., Salah, R., Bakour, R., Drouiche, N., Abdi, N., Grib, H., Lounici, H., Mameri, N. (2015). Effects of chitin and its derivatives on human cancer cells lines. *Environmental Science Pollution Research, 22,* 15579–15586.

Dash, M., Chiellini, F., Ottenbrite, R.M., Chiellini, E. (2011). Chitosan - A versatile semi-synthetic polymer in biomedical applications. *Progress in Polymer Science, 36,* 981–1014.

FAO. (2006). The State of Fisheries and Aquaculture; Fisheries and Aquaculture Department Food and Agriculture Organization of The United Nations Rome, available at http:// www.fao.org/docrep/009/A0699e/A0699e00.htm.

Gokoolu, N., Yerlikaya, P. (2003). Determination of proximate composition and mineral contents of blue crab (*Callinectes sapidus*) and swim crab (*Portunus pelagicus*) caught off the Gulf of Antalys. *Food Chemistry, 80,* 495–498.

Karakoltsidis, P.A., Zotos, A., Constantinides, S.M. (1995). Composition of the commercially important mediterranean finfish, crustaceans and molluscs. *Journal of Food Composition and Analysis, 8,* 258–273.

Khor, E., Wan, A.C.A. (2013). Chitin: Fulfilling a Biomaterials Promise, 2nd ed.; Elsevier Ltd.: Waltham, MA, USA.

Kim, K.Y., Nam, K.A., Kurihara, H., Kim, S.M. (2008). Potent _-glucosidase inhibitors purified from the red algae *Grateloupia elliptica*. *Phytochemistry*. *69,* 2820–2825.

Matet, M., Heuzey, M.-C., Ajji, A. (2014). Morphology and antibacterial propertiesof plasticized chitosan/metallocene polyethylene blends. *Journal of Materials Science, 49*(15), 5427–5440.

Mathew, S., Ammu, K., Viswanathan, P.G., Devadasan, N.K. (1999). Cholesterol content of Indian fish and shellfish. *Food Chemistry, 66,* 455–461.

Rinaudo, M. (2006). Chitin and chitosan: properties and applications. *Progress in Polymer Science, 31*(7): 603–632.

Synowiecki, J., Al-Khateeb, N.A.A.Q. (1997). Mycelia of *Mucor rouxii* as a source of chitin and chitosan. *Food Chemistry, 60*(4): 600-610.

Takiguchi, Y. (1991). Physical properties of chitinous materials. In: R.H. Chen, & H.C.Chen (Eds.), Advances inchitin science. Proceedings from the third Asia–Pacific Chitin. Chitosan Jikken manual. Japan:Gihodou Shupan Kabushki Kasisha *3,* 1–7 pp.