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Eco-friendly extraction and characterization of chitosan encapsulated nanoparticles for anti-diabetic application

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Abstract
The aim of this study was to examine the anti-diabetic effect of the plant-encapsulated chitosan nanoparticles which was obtained from the shell and plant samples that are organic waste utilized in the process of preparing a biocompound that can be converted as a bio-product (plant coated chitosan nanoparticles). This biocompound is economic, eco-friendly, cost-effective, non-toxic in nature and also less time consuming for its preparation. The plant-coated chitosan nanoparticles was used for the *in vitro* α-glucosidase inhibition anti - diabetic assay and *in vitro* α- amylase inhibition anti - diabetic assay. The anti-diabetic activity of encapsulated chitosan nanoparticles was found to be positive as there was good inhibition of α-glucosidase and α-amylase. Therefore, the plant-encapsulated chitosan nanoparticles can be used in drug delivery systems thereby leading the way to synthesize novel pharmaceutical compounds.

Keywords: Chitosan, nanoparticles, *in-vitro* α-glucosidase inhibition anti-diabetic assay, *in-vitro* α-amylase inhibition anti-diabetic assay

Introduction
Nanobiotechnology is a recently developed research field that deals with configuring, synthesizing, and applying nanoparticles that range from 1 to 100 nm (Ratan *et al*., 2020) [13]. Nanomedicine is an application of nanotechnology that works in the field of health and medicine. Nanomedicine is a branch of medicine that is widely used in the field of medicine and health as the drug delivery systems are made up of nano-scale particles or molecules that can enhance the drug bioavailability. (Nikalje, 2015) [12].
Diabetes Mellitus is a commonly occurring chronic metabolic which is defined by sustained hyperglycemia. It can be caused by either a low systemic insulin level or insulin resistance or both (Burgess *et al*., 2021) [1]. According to WHO's statistics, currently more than 422 million people are living with diabetes worldwide and the mortality rates due to diabetes have increased by 70% globally between 2000 and 2019. Therefore, diabetes is considered a major threat in today's scenario. Diabetes is a major reason for systemic conditions like diabetic retinopathy, renal failure, cardiovascular diseases, stroke and limb amputation. It is a chronic disorder that occurs either when there is low insulin production by the pancreas or when the body cannot use the produced insulin. The intestinal absolute deficiency of insulin secretion is associated with enzymes such as α -amylase and α -glucosidase. These two enzymes play a key role in the breaking down of starch and oligosaccharides to glucose. The suppression of these two enzymes leads to a delay in the glucose absorption in the intestine therefore controlling the postprandial blood sugar (Das *et al*., 2017) [3].
Chitin has a variety of compounds that can be used as functional properties of interest in the nutraceutical and medical fields. Chitin can be obtained from the exoskeletons of crustacean biomass that have been wasted in fishing extractive and processing activities. This promotes the sustainability of this sector and leads to its development under a bio-economical framework. Crustacean biomass is one of the main sources of this nontoxic polysaccharide available for commercial use (Lopes *et al*., 2018) [7]. Emerita asiatica (mole crab) is found in the intertidal regions. The smallest species are dominantly found in fine sand near high water marks and the largest species are found in coarse sand near low water marks.
Cucumis melo var. agrestis (field muskmelon) is a weed that belongs to the family of *cucurbitaceae* (Sohrabikertabad *et al*., 2013) [15]. *Cucumis melo* has many phenolic compounds, functional properties and also tasty and juicy fruit with nutritive and medicinal values that make it one of the most consumed 37 fruit crops worldwide (Mallek *et al.*, 2017) [9].

Chitin is partially or fully deacetylated to form chitosan (Cazón *et al*., 2017) [2]. Chitosan nanoparticles is the most widely used biodegradable nanoparticle as of now due to their therapeutic properties such as antidiabetic agents, wound healing, antimicrobial agents and effective drug delivery control (Subramaniam *et al*., 2022) [16]. Chitosan is considered a nontoxic polysaccharide that can be used as a permeation enhancer for hydrophilic molecule absorption. Chitosan has the capacity to adhere to the mucosal surface and it can open up the tight junction between epithelial cells. Claudin-4 which is a transmembrane protein that is responsible for tight junction integrity is chitosan mediated, so developing a stable and efficient chitosan-based insulin delivery system has been more effective (Ma *et al*., 2005) [8]. Chitosan nanoparticles is biodegradability, biocompatibility, has better stability, low toxicity with easy preparation methods. These properties make chitosan a vital tool for novel drug delivery systems (Nagpal *et al*., 2010) [10]. The anti-diabetic activity of chitosan nanoparticles administered to the mice reduced the glucose level, total cholesterol level and triglyceride levels and increases the insulin level as compared to the commercially powdered chitosan. (Seo *et al*., 2010) [14].

The current study aimed to formulate the efficacy of plant-encapsulated chitosan nanoparticles as an effective alternative for targeted drug delivery and diabetes treatment. The bio-product was made of plant and animal species that are taken from organic wastes (biomass).

Materials and Methods

The animal sample *Emerita asiatica* (Mole crab) was collected in Marina beach, East coast region, Chennai, Tamil Nadu. The plant sample *Cucumis melo var agrestis* (Wild melon or Field muskmelon) was collected in a fallow land from Alampoondi village in Vilupuram, Tamil Nadu. It was washed with distilled water, shade-dried and powdered.

Synthesis of chitosan nanoparticles was done by demineralization, deproteinization and decolourisation. Then the encapsulation of chitosan nanoparticles was done with the help of a sonicator and electrical magnetic stirrer for the beads to form which as were collected and dried.

In vitro anti - diabetic activity

1. α-glucosidase inhibition activity

50 L of 50 mM phosphate buffer, 10 µL of α-Glucosidase and 20 µL of different concentrations of synthesized sample i.e. 1 µgm, 10 µgm, 30 µgm, 100 µgm, 125 µgm, 150 µgm, 200 µgm, 250 µgm were taken. Then it was incubated at 37 °C for 5 minutes. Then add 20 µL of 1 Mm p-nitrophenyl-α-d-Glucoside (PNPG) and incubation was done at room temperature (37 °C) for 30 minutes. Then add 50 µL of 0.1 mM sodium carbonate to stop the reaction. Absorbance was read at 405 nm.

2. α-amylase inhibition activity

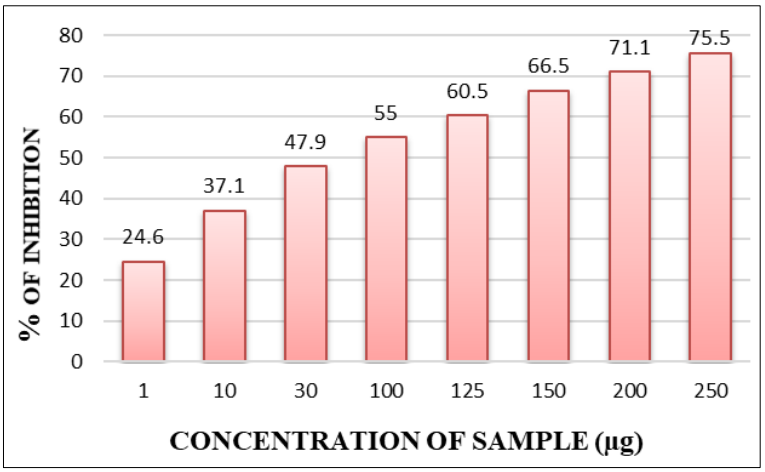
0.5 ml of different concentrations of synthesized sample i.e. 1 µgm, 10 µgm, 30 µgm, 100 µgm were taken. To that 0.5 ml of 0.2 mM PBS and 0.5 ml of α-amylase enzyme were added. Incubation was done at 25 °C for 10 minutes. Then 0.5 ml of 1% starch solution was added. It was kept under incubation at 25 °C for 10 minutes. Then 1 ml of DNS (3,5-dinitrosalicylic acid) solution was added and kept in a boiling water bath for 5 minutes. After that, the heating kit was cooled at room temperature (37 °C). Then the absorbance was read at 540 nm.

Plant encapsulated chitosan nanoparticles

Results

In α-glucosidase assay for 1 µg of sample the percentage of inhibition was 24.6, for 10 µg of sample the percentage of inhibition was 37.1, for 30 µg of sample the percentage of inhibition was 47.9, for 100 µg of sample the percentage of inhibition was 55, for 125 of sample the percentage of inhibition was 60.5, for 150 of sample the percentage of inhibition was 66.5, for 200 of sample the percentage of inhibition was 71.1, for 250 of sample the percentage of inhibition was 75.5.

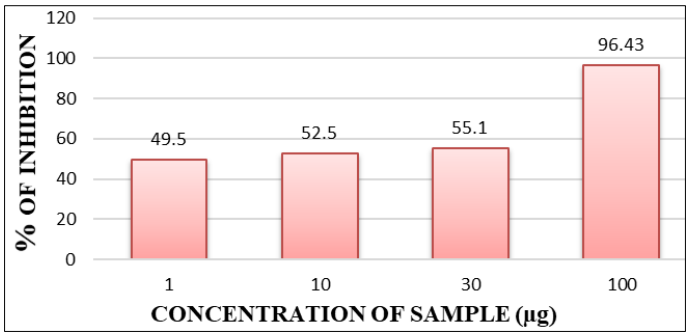
In α- amylase assay for 1 µg of sample the percentage of inhibition was 49.50, for 10 µg of sample the percentage of inhibition was 52.50, for 30 µg of sample the percentage of inhibition was 55.10, for 100 µg of sample the percentage of inhibition was 96.43.



Concentration of Sample (µg)

α- glucosidase inhibition activity of encapsulated chitosan nanoparticles

S. No	Concentration of sample (µg)	% Inhibition of α-glucosidase
1.	1	24.6
2.	10	37.1
3.	30	47.9
4.	100	55
5.	125	60.5
6.	150	66.5
7.	200	71.1
8.	250	75.5



Concentration of sample (µg)

α-amylase inhibition activity of encapsulated chitosan nanoparticles

S. No	Concentration of sample (µg)	% inhibition of α- amylase
1.	1	49.50
2.	10	52.50
3.	30	55.10
4.	100	96.43

Discussion

The manipulation ability of nanoparticles on molecules and their structures has made far-reaching changes in the drug delivery system. The chitosan nanoparticles have been used as a key application in parental drug delivery, oral administration of drugs, non-viral gene delivery, vaccine delivery, stability improvement of drug, in tissue engineering and in the effective delivery of insulin (Nagpal *et al*., 2010) [10].

Chitosan is a linear biopolymer that is synthesized by d-glucosamine and n-acetyl-d-glucosamine. Chitosan and its derivatives are found to have several biological properties such as antioxidant, antihypertensive, anti-inflammatory, anti-coagulant, anti-tumoral, anti-microbial, hypocholesterolemic and anti-diabetic effects (Ngo and Kim, 2014) [11]. Due to their high biodegradability, biocompatibility and their modification abilities, chitosan nanoparticles are being used as drug-delivery carriers (Li *et al*., 2018) [5].

The plant associated bio-polysaccharides has many advantages such as widespread accessibility from nature as plant-based sources are readily available, sustainable production, availability of easy and cost-effective extraction methodologies, aqueous solubility, non-toxicity and biodegradability (Guru *et al*., 2023) [4]

Enzymes such as α-glucosidase and α-amylase are the main enzymes involved in the metabolism of carbohydrates. α-amylase degrades complex dietary carbohydrates into oligosaccharides and disaccharides, which is then converted into monosaccharides by α -glucosidases. Then this glucose is then absorbed by the gut and results in postprandial hyperglycemia. Inhibition of these two enzymes α -glucosidases and α -amylase can limit postprandial glucose levels by delaying the process of carbohydrate hydrolysis and absorption, making such inhibitors useful in the management of type 2 diabetes mellitus (Liu *et al*., 2013) [6]. The anti-diabetic activity of encapsulated chitosan nanoparticles was found to be positive as there was good inhibition of α-glucosidase and α-amylase. Similarly in studies conducted by (Seo *et al*., 2010) [14] it is observed that feeding the nano powdered chitosan to the mice can reduce the glucose, total cholesterol level, and triglyceride concentration, and can increase the insulin level.

In vitro α-glucosidase and α-amylase activities results reveal that the synthesized plant encapsulated chitosan nanoparticles have a significant α-glucosidase and α-amylase inhibition activity. Inhibition rate of α-glucosidase and α-amylase was based on the dose rate of sample. As the dose rate of sample increased, the inhibition rate was higher. Therefore, this product can be an alternative to reduce the use of harmful chemical compounds and also it is harmless to the environment.

Conclusion

Nano-pharmaceuticals are an emerging field where nanoparticles are used for therapeutic drug delivery system. This research focuses on the alarmingly increasing rate of diabetes in Indian scenario affecting large population. Therefore, there is a need to develop a bioactive compound for beneficial technology in nano- biomedicine. The shell and plant samples that are organic waste can be utilized in this process of preparing a biocompound and can be converted as a bio-product (plant coated chitosan nanoparticles) which is economic, eco-friendly, cost effective, non-toxic in nature and less time consuming for its preparation. Chitosan nanoparticles has biodegradability, biocompatibility, better stability, low toxicity properties so this organic nanoparticle

can be an alternative to harmful chemical compounds. The *in vitro* anti - diabetic assay shows good inhibition of the enzymes α-glucosidase and α-amylase which is a good property of being a valuable tool for novel drug delivery systems.

References

1. Burgess JL, Wyant WA, Abujamra AB, Kirsner RS, Jozic I. Diabetic wound-healing science. Medicina. 2021;57(10):1072.
2. Cazón P, Velazquez G, Ramírez JA, Vázquez M. Polysaccharide-based films and coatings for food packaging: A review. Food Hydrocolloids. 2017;68:136-148.
3. Das MP, Rebecca LJ. Characterization of antidiabetic activity of Silver Nanoparticles using aqueous solution of *Ficus glomerata* (fig) gum. International Journal of Pharmacy and Biological Science. 2017;8(2):424-429.
4. Guru PR, Kar RK, Nayak AK, Mohapatra S. A comprehensive review on pharmaceutical uses of plant-derived biopolysaccharides. International Journal of Biological Macromolecules; c2023. p. 123454.
5. Li J, Cai C, Li J, Li J, Li J, Sun T, *et al*. Chitosan-based nanomaterials for drug delivery. Molecules. 2018;23(10):2661.
6. Liu J, Lu JF, Kan J, Jin CH. Synthesis of chitosan-gallic acid conjugate: structure characterization and *in vitro* anti-diabetic potential. International journal of biological macromolecules. 2013;62:321-329.
7. Lopes C, Antelo LT, Franco-Uría A, Alonso AA, Pérez-Martín R. Chitin production from crustacean biomass: Sustainability assessment of chemical and enzymatic processes. Journal of Cleaner Production. 2018;172:4140-4151.
8. Ma Z, Lim TM, Lim LY. Pharmacological activity of peroral chitosan–insulin nanoparticles in diabetic rats. International Journal of pharmaceutics. 2005;293(1-2):271-280.
9. Mallek-Ayadi S, Bahloul N, Kechaou N. Characterization, phenolic compounds and functional properties of *Cucumis melo* L. peels. Food chemistry. 2017;221:1691-1697.
10. Nagpal K, Singh SK, Mishra DN. Chitosan nanoparticles: a promising system in novel drug delivery. Chemical and Pharmaceutical Bulletin. 2010;58(11):1423-1430.
11. Ngo DH, Kim SK. Antioxidant effects of chitin, chitosan, and their derivatives. In Advances in food and nutrition research. Academic Press. 2014;73:15-31.
12. Nikalje AP. Nanotechnology and its applications in medicine. Med chem. 2015;5(2):081-089.
13. Ratan ZA, Haidere MF, Nurunnabi MD, Shahriar SM, Ahammad AS, Shim YY, *et al*. Green chemistry synthesis of silver nanoparticles and their potential anticancer effects. Cancers. 2020;12(4):855.
14. Seo MH, Park JH, Kwak HS. Antidiabetic activity of nanopowdered chitosan in db/db mice. Food Science and Biotechnology. 2010;19(5):1245-1250.
15. Sohrabikertabad S, Ghanbari A, Mohassel M, Mahalati MN, Gherekhloo J. Effect of desiccation and salinity stress on seed germination and initial plant growth of Cucumis melo. Planta Daninha. 2013;31(4):833-841.
16. Subramaniam S, Kumarasamy S, Narayanan M, Ranganathan M, Rathinavel T, Chinnathambi A, *et al*. Spectral and structure characterization of Ferula assafoetida fabricated silver nanoparticles and evaluation of its cytotoxic, and photocatalytic competence. Environmental Research. 2022;204:111987.