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In-vitro Anti-Diabetic Activity from Poultry Waste

C.Divya*, K.Dhanalakshmi

Manonmaniam Sundaranar University, Tirunelveli, India

Abstract : Diabetes is a metabolic disease in which the person has high blood glucose due to lack of insulin secretion. Patient may lack of keratin. The damage of epithelial cells is protected using keratin. It is the key structural material making up the outer layer of human skin. Protein helps to secrete insulin. Chicken feathers have elevated keratin protein content and can be a suitable protein source. The feather is composed of about 90% of keratin, which has a structure characteristic of materials of high mechanical strength. In this work, keratin is extracted from Chicken feathers. The keratin should be mixed with saturated fat for easy absorbent of vital protein. The keratin property can be characterized using FTIR. To confirm the properties of anti diabetic, in vitro studies were carried out and confirmed the anti diabetic property.
Keywords : Anti-Diabetics, Keratin, chicken feather, Amylase activity.

I. Introduction

Chicken Feathers has high protein content. Huge quantity of feathers is produced in poultry slaughterhouse[3-8]. The keratin exists in α-helix conformation in feather wool, and as pleated sheet in barbs and rachis. Chicken feather contains 91% of keratin. The keratin extracted from feather has a high content of the amino acids glycine, alanine, serine, cysteine and valine, but lower amounts of lysine, methionine and tryptophan[9-15]. Currently, feather treated with microbial keratinase is attracting wide attention with several applications. Many people are suffering from diabetics. Diabetic is the chronic disease due to the disorder of carbohydrate, fat and protein metabolism characterized by increased blood sugar level. Treatment of diabetics by allopathic is costly. Recently, natural products are used to treat the diabetic in a cost effective manner. Keratin is used to treat diabetic. Bauhinia Purpurea [1] stem bark for anti diabetic and anti inflammatory is reported. Dried petroleum ether and hexane extracts of stem bark of bauhinia purpurea were analyzed for in-vitro anti diabetic activity. The extracts shows high level of activity and high concentration was high effective as an anti diabetic agent. Green synthesis of zinc oxide nanoparticle [2] from hibiscus with small size of zinc oxide nanoparticle with Streptozotocin induced diabetic mice has high anti-diabetic activity. ZnO induces the function of Th1,Th2 cells and other genes of pancreas related with diabetes.

II. Experimental

Pre-treatment of the feathers

Chicken feathers are collected from poultry around Tirunelveli and soaked in ether for 24 hr. soaked feather is washed with soap oil and double distilled water. The main purpose is to clean the feathers from stains, oil and grease before processing it. Cleaned feather is then dried in shadow and chopped into fine pieces for further extraction.

Keratin Extraction

The chopped feather is dissolved in 5% NaOH solution as in fig.1(A) and it was incubated for 4hrs at 40 ºC as shown in fig.1 (A). Incubated feathers are filtered by using Whatman filter paper as shown in fig.1(C). The collected Filtrate was lyophilized and stored. The presence of keratin is tested by Biuret method.

A)

B)

C)

Fig.1 : Keratin Extraction

Biuret method

The Biuret reagent was prepared by adding 3 g of $CuSO_4 \cdot 5H_2O$ and 9 g of sodium potassium citrate to 500 mL of 0.2 N NaOH solution, followed by the addition of 5 g of KI. The resulting solution was then brought to a total volume of 1 L with 0.2 N NaOH

III. Characterization of keratin

Fourier Transform Infrared Analysis

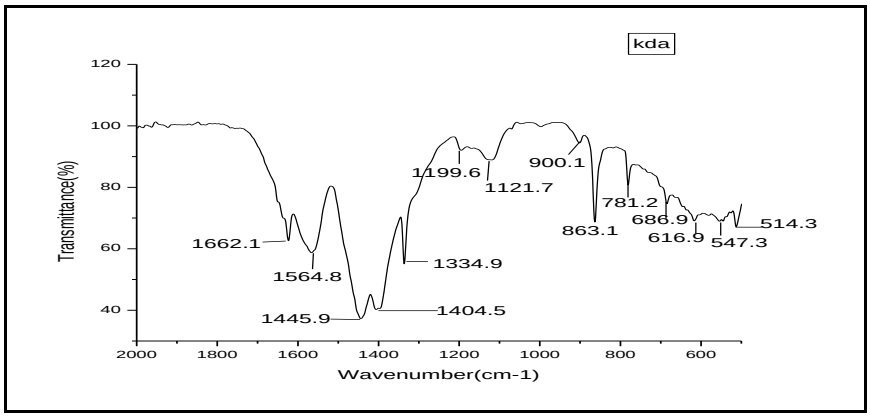

Fig.2. FTIR Spectrum

The changes in the functional groups of the hydrogels that may have been caused by the physical or chemical treatments were evaluated using Fourier transform infrared spectroscopy (FT-IR, Varian 1000 FTIR Scimitar series, PIKE Technologies, USA) on freeze dried samples as shown in figure 2.

IV. In-vitro studies of Anti-diabetic Activity

The inhibition assay was performed using the chromogenic DNSA method [Miller, 1959]. In this alpha amylase method, 2 ml of 0.05 M sodium phosphate buffer at PH 6.9, 50 µl of alpha amylase enzyme, 1 ml of drug solution at different concentrations were incubate at 37°C for 10 min. then 500 µl of 1% (w/v) starch solution in the prepared buffer is added to each concentrations and incubated at 37°C for 15 min. After incubation 1.0ml of DNSA reagent was added and kept in the water bath for few minutes. Absorbance is measured at 540 nm after cooling at room temperature. The maltose liberated was determined by the help of standard maltose curve and activities were calculated according to the following formula and the % of inhibition is calculated as shown in table 1.

$$\text{Activity} = \frac{\text{Conc. of Maltose liberated X ml of enzyme used}}{\text{Mol. wt of maltose X incubation time (min)}} \text{ X dilution factor}$$

One unit of enzyme activity is defined as the amount of enzyme required to release one micromole of maltose from starch per min under the assay conditions. The inhibitory/induction property shown by the sample was compared with that of control and expressed as percent induction/inhibition. This was calculated according to the following formula

$$\%\ \text{inhibition/induction} = \frac{\text{Activity in presence of compound}}{\text{Control Activity}} \text{X } 100$$

V. Results and Discussion

The in-vitro alpha amylase inhibitory studies demonstrate that keratin from the chicken feather has a well anti diabetic activity. The percentage inhibition at 100,200,300,400,500,1000,1500,2000 µg/ml concentrations of keratin shows concentration dependent reduction in percentage inhibition. The Sample has

shown maximum inhibition of amylase 81% at highest 2000 µg/ml concentration as shown in table 1. As a result keratin shows significant activity when compared to acarbose standard drug as shown in figure 2.

Table 1:Amylaze Inhibition Analysis

Sample	OD@540 nm	Maltose liberated (µg)	Activity (µmoles/ml/min)	% activity	% inhibition
Control	1.881	154.1667	0.04278723	100	0
100 µg	1.659	135.6667	0.037652762	88.00	12.00
200 µg	1.461	119.1667	0.033073372	77.30	22.70
300 µg	1.081	87.5	0.024284644	56.76	43.24
400 µg	0.871	70	0.019427715	45.41	54.59
500 µg	0.653	51.83333	0.01438576	33.62	66.38
1000 µg	0.583	46	0.012766784	29.22	70.78
1500 µg	0.479	37.33333	0.010361448	23.72	76.28
2000 µg	0.39	29.91667	0.008303035	19	81

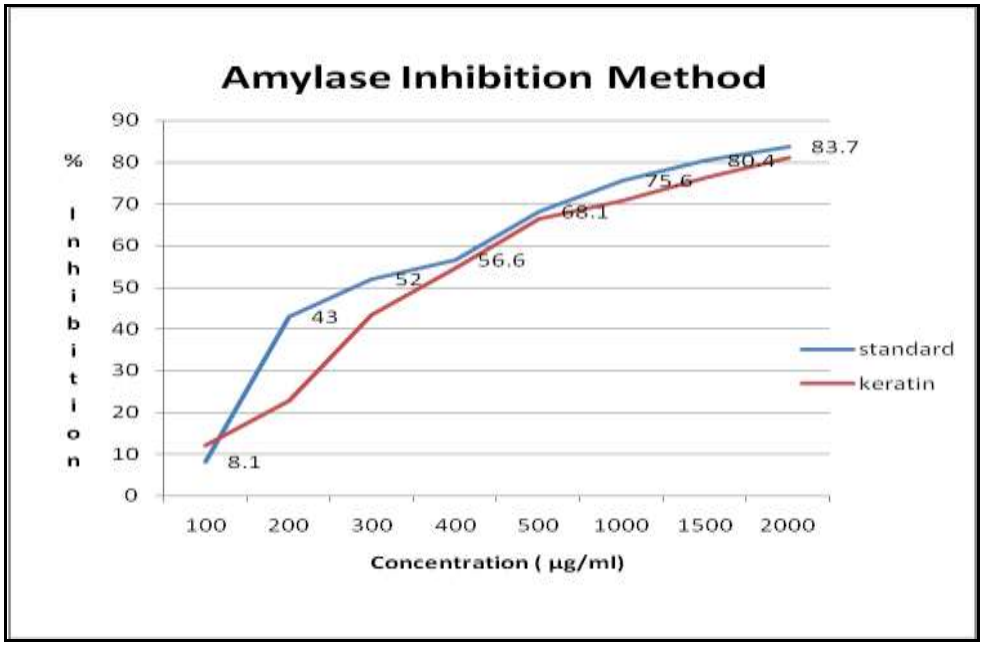

Fig.2. Alpha amylase Inhibition Assay of Keratin

VI. Conclusion

Synthesis of keratin from chicken feather was found to have antidiabetic activity. Usage of chicken feather leads to waste management in poultry industry since the chicken feathers consist of 90% crude protein and poses an environmental problem due to its time consuming decomposition. The chicken feathers were first dissolved using reducing agents and protein precipitated out from the solution using sodium hydroxide. The presence of the protein was confirmed first by the biuret test where the reagent changed to purple color in the presence of peptide bonds. The FTIR (fourier transform infrared spectroscopy) also confirmed the presence of amino and a carboxyl group in the sample, the two groups confirms the presence of amino acids. Alpha amylase method shows significant in vitro anti diabetic activity against keratin obtained from feather.

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