

Stabilization of Collagen by Its Interaction with Tannin Extracted from *Punica Granatum*

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Abstract: Collagen is a protein found in most connective tissues, including cartilage, of higher animals. Collagen is the main structural protein of the various connective tissues in animals [1]. It has extensive application as biocompatible biomaterial in wound healing, as drug carrier, cosmetics, etc. Therefore the following study is done to stabilize, type 1 collagen of fish skin and scales using plant poly phenols. The comparison of tensile strength of native and treated collagen films will display the resultant changes in stability. Fish skin and scales are general waste material; therefore their utilization can lead to development of low economical methods for wound healing purposes. The source of collagen is *Channa Striatus*; a predatory fresh water fish, indigenously famous for benefits in treating wound and pain and in boosting energy of the sick. Its extracts can also be used for the formation of bioactive molecules. Source for the extraction of tannins, the polyphenol is pomegranate peel (*Punica granatum*).

Key words: SDS page, collagen extraction, standardization with gallic acid

I. INTRODUCTION

Collagen type 1 is a protein found in most connective tissues of higher animals providing strength and support. It comprises of polypeptide chains that are rich in hydroxyproline amino acids and are twisted together to form triple-helical structure. Over 20 different type of collagen have been identified; the main types are I, II and III, which make up 80% of the body's collagen. Type I and III are important for wound healing. It also plays a role in wound healing due to its chemotactic role. It attracts cells such as fibroblasts and keratinocytes to the wound. This encourages debridement, angiogenesis, and re-epithelialisation. Artificial Collagen dressings provide alternate collagen source to decrease the protease activity (matrix metalloprotease) on native collagen. Therefore, there is a need to stabilize the collagen for wound healing purposes. This can be achieved by use of polyphenols extracted from plant sources. Phenols and poly phenols are produced as secondary metabolites from various plant sources and tannin is one of them. Tannin is present in bark, wood, leaves, fruit, roots and seeds. Highly purified tannic acids can lead to reduction of scar tissue formation due to its powerful antibacterial and angiogenic activity.

II. MATERIALS AND METHODS

A. Sample

Channa striatus, a predatory fresh water fish was obtained from local market. The outer skin was removed and cut into small pieces used freshly for extraction. The scales were properly washed and used freshly for extraction.

B. Preparation of collagen from the outer skin

All the procedures for collagen extraction were performed at 4°C. The skin was washed with 0.1 M NaOH to remove non-collagenous proteins and was then washed with distilled water. The washed skins were then kept in 0.5M Acetic acid for 3 days and homogenized. The homogenized sample was then mixed and salted out by adding 5% NaCl. The precipitate is separated out and dissolved in 0.5M Acetic acid and dialyzed.

C. SDS –polyacrylamide gel electrophoresis (SDS-PAGE)

SDS page was used to analyze different protein samples to check its purity. Samples were dissolved in buffer containing SDS for reducing disulfide bridges and bromophenol blue as tracking dye. Samples were heated at 70°C for 15min. The stacking gel, pH 6.8 and separating gel, pH 8.8 of 7% acrylamide were used. After electrophoresis the gels were stained with staining solution containing Coomassie Brilliant Blue R-250 for 1hr. Destaining solution was used to destain the dye.

D. Extraction of tannin from plant material

Samples used were the hard covering of *Punica granatum*. The extraction was done using 80% methanol. The outer covering of pomegranate was weighed out (10g) and kept in amber bottles with 100ml of 80% of methanol. Extraction procedure was repeated. All the extracts were filtered through filter paper and evaporated to concentrate the sample.

E. Ferric chloride test:

Few drops of ferric chloride was added to a small amount of the sample. Presence of dark green or blue colour indicated the presence of tannins.

F. Folin's Test

Tannin obtained from *Punica granatum* was estimated using Folin-Dennis method [7]. Standardization was done using Gallic acid and tannin concentration was expressed as units of Gallic Acid.

TABLE 1: Test for follin

Sample	Sodium carbonate solution (0.55M)	Folin's reagent (1N)
0.5ml	2.5ml	0.25ml

G. Gelatin precipitation method

1% gelatin was prepared in 0.9% NaCl. To 5ml of 1% gelatin solution, 1ml of tannin solution was added and incubated for 30min. The interaction of tannin with 1% gelatin led to the formation of white precipitate. [Fig2]

H. The interaction of tannin with collagen

25 ml of dialyzed samples of protein was taken and spread onto a non-stick plastic container. It was kept for drying in desiccator for 1 day. A thin film was formed, which was taken out and was treated with tannin extract.

Where RC is Rat collagen, Sample A and D is dialyzed collagen of fish scale and sample B and C is dialyzed collagen of fish skin. [Fig1] and [Fig4]

III. RESULTS

RC	D	C	B	A
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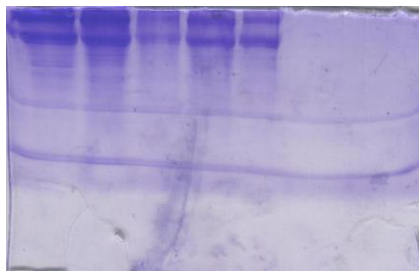


Fig1: SDS- polyacrylamide gel electrophoresis

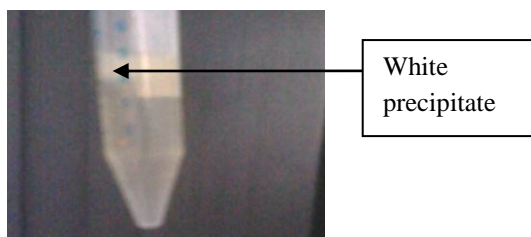


Fig2: Formation of white precipitate using gelatin precipitation method

TABLE 2: Standardization with gallic acid

Concentration (mg/ml)	O.D at 660 nm
Blank	0.00
0.005	0.05
0.0075	0.08
0.01	0.13
0.025	0.28
0.05	0.59
0.075	0.65
0.1	1.00

Therefore according to the standard graph, the concentration of tannin in extract is 0.35 Units of Gallic Acid. [Fig3]

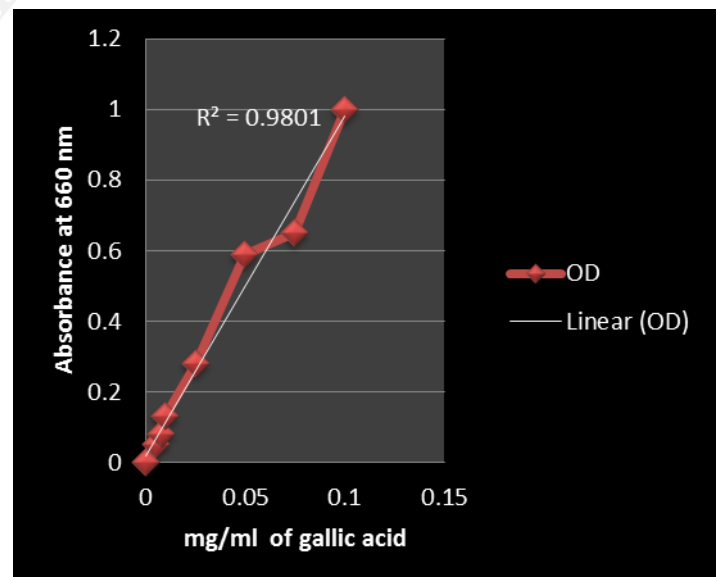


Fig 3: Standard Graph

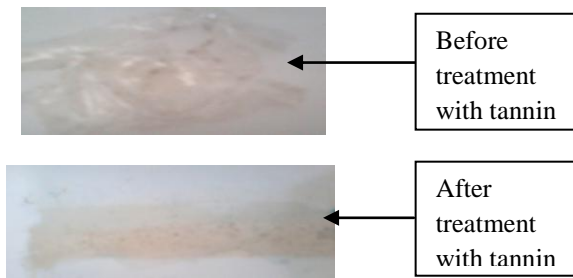


Fig 4: Interaction of tannin with collagen

IV. DISCUSSION

1. The SDS gel electrophoresis showed the purity of collagen extracted. Three bands are clearly visible at high molecular weight region which is a peculiar feature of collagen.
2. The interaction of tannin with gelatin gave a white precipitate. This shows the presence of protein interacting tannins in the plant extract. The formation of tannin-precipitant complex is primarily by hydrogen bonding.
3. The collagen matrix that is not treated with tannin is unstable at room temperature. It deforms quickly. But the matrix that is treated with tannin is stable in comparison to untreated one which should be confirmed by measuring tensile strength of collagen.

V. CONCLUSION

Physically collagen seems to be more stable when treated with tannin than untreated collagen. Tensile strength should be measured for the collagen films, both the treated and untreated, for further confirmation.

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