Research article

Extraction and Characterization of Fish Collagen for Enhanced Wound Healing Activity on A549 Cell Lines

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Abstract

Keywords

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antimicrobial activity;

wound healing assay

Collagen is a proteinaceous material that is present abundantly in animal tissues and gives physical strength and stability to the tissues. Collagen extracted from animal tissues has been applied in the biomedical and healthcare sectors. In recent days, aquatic organisms have shown great potential for collagen extraction and purification. In the present study, we selected three fish species: *Naucrates ductor, Oreochromis mossambicus* and *Cyprinus carpio*for collagen extraction from both their skin and scales. The biomass was pre-treated to remove any non-collagenous matter and fat. Acid-soluble collagen was extracted using acetic acid and a maximum extraction of 51.55±1.02% was reported. The total protein fractions and the denaturation temperatures of the collagen extractswere measured. The molecular weights and the subunit compositions of the collagen extracts were analyzed using SDS-PAGE and the presence of two α chains, one β, and one γ chain. Amino acid profiling was done in an amino acid analyzer after hydrolysis and about one third of the total amino acid content was glycine. The solubility characteristics of the collagenextracts were analyzed at different ranges of pH and salinity. The antimicrobial activities of the collagen extracts were examined against four bacterial species, and it was proven that skin-derived collagens had greater antibacterial potential than extracts of the scales. *In vitro* studies on the wound healing capacity of the extracted collagen were performed using A549 cell line cultured in F-12K medium, and 95% of cell regeneration was observed in collagen-supplemented dispersed cell lines.

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1. Introduction

Vast coastal line of the Indian Ocean and indigenous technologies are not just making India one of the largest producers of fish and other marine foods, but also unveiling a huge scope for the extraction of biomolecules of medicinal importance from marine sources. Collagen is one such product that possesses a great number of applications in the healthcare sector. The extracellular matrix of terrestrial animals is predominantly constituted by the fibrous protein collagen which plays an important role in the physical strength and integrity of tissue structures. About one-fourth of all animal proteins belong to the family of collagen protein [1]. Global outbreaks such as bovine spongiform encephalopathy (BSE), foot-and-mouth disease (FMD), and transmissible spongiform encephalopathy (TSE) that occurred the past decades cast doubt on the safe usage of animal-derived collagen and emphasized the need for finding alternative source for collagen [2]. India being the largest fish producer means that the country hasmany opportunities to turn fish wastes into useful products such as collagen. Collagen has a wide variety of applications in different fields like wound healing, tissue engineering, and drug delivery [3].

Fish collagen is highly porous with interconnecting pore structures, and it allows the passage of oxygen and other nutrients that are essential for the wound healing process. Its great stability and minimum immunological response make fish collagen membrane an ideal candidate for wound healing [4]. Collagen-based hydrogel formulations have a significant effect on fibroblast production by enhancing collagen deposition in the wound region. They can create a perfect environment comparable to the extracellular matrix, which is important for fibroblast production [5]. The rapid proliferation and migration of fibroblasts along with keratinocytes speed up tissue remodeling and recovery [6]. Nanofibers fabricated by incorporating collagen and zinc oxide nanoparticles demonstrate good cytocompatibility, enhance the adhesion potential of fibroblast cells, and can be used as scaffolds to support the rapid wound healing process [7]. Hydrogels containing nano-encapsulated curcumin fabricated from chitosan nanoparticles enhanced collagen deposition in subcutaneous and skin wound models on Wistar albino rats and increased wound healing efficiency [8, 9]. Marine-based collagen derivatives are used extensively in the biofabrication of organs like bone and cartilage using advanced techniques like 3D bioprinting and deliver vital breakthroughs in the fields of regenerative medicine and tissue engineering [10].

Scaffolds of fish collagen show extensive cell viability and can be used as an outstanding material in both hard and soft tissue applications. Collagen hydrogels along with some proteins or polymers were used in the regeneration of nerve cells [11]. The derivatives of collagen protein such as gelatin are used extensively in the pharmaceutical industry for capsule formulation, tablet coating, and emulsion stabilization. Because of their high biocompatibility, collagen and its derivatives, and especiallythose obtained from cold water fish are used in drug delivery as they can release drugs rapidly and precisely at the target site [12]. In this study, three different fish species for collagen extraction and characterization are used. Molecular weight, subunit composition, and amino acid composition of the extracts were analyzed, and the antimicrobial activities of the collagen extractswere measured against bacterial pathogens. Finally, wound healing assays were performed on animal cell lines.

2. Materials and Methods

2.1 Biomass collection and pre-processing

Naucrates ductor, Oreochromis mossambicus, and *Cyprinus carpio* fishes were procured from Chengalpattu market, Tamil Nadu, India. The fish were washed thoroughly with distilled water,

and the skin and scales were peeled off from the fish using a sharp knife. The skin and scales of the fish were individually mixed with 0.1N NaOH in 1:10 (w/v) ratio and stirred for 48 h in order to remove any non-collagenous materials. Each sample was thenfiltered and washed using distilled water until the wash water reached pH 7. Next, each neutralized sample was mixed with 10% butyl alcohol at 1:10 (w/v) ratio and stirred gently for 24 h to solubilize the fat present in the sample. The fat-free samples were then washed with distilled water to remove the alcohol fraction [13].

2.2 Collagen extraction

Pre-processed samples of both fish scales and skin were separately soaked in 0.5M acetic acid (1:15 w/v ratio) and shaken for 72 h. The mixtures were filtered, and the filtrates were centrifuged at 10,000rpm for 15 min at 4°C. The process was repeated for two more cycles and all the supernatants were combined together. The supernatants were salted out using 2.6M NaCl to precipitate the protein. The precipitates were centrifuged at 10,000rpm for 30 min, using a refrigerated centrifuge. The pelleted precipitates were dialyzed and excess salt was removed from them. The dialyzed precipitates obtained from both skin and scale were freeze-dried and stored for further analysis. The concentrations of collagen in the extracted liquids were measured gravimetrically and recorded [14]. All the experiments were performed in triplicate and mean values were used.

2.3 Characterization of collagen

2.3.1 Total protein content

The total protein content of the collagen extracts was calculated by Lowry's method withbovine serum albumin as the standard [15].

2.3.2 Molecular weight

The molecular weight of collagen was predicted by performing SDS-polyacrylamide gel electrophoresis using 5% stacking gel and 7.5% resolving gel. The gel was loaded with a molecular weight marker and the bands produced by the collagen were compared with the standard. After electrophoresis, the gel was stained using Coomassie Brilliant Blue R-250 [16].

2.3.3 Amino acid profiling

Collagen samples were subjected to hydrolysis with 4% methane sulfonic acid at 115°C for 24 hunder reduced pressure conditions. The hydrolysate was added with an equal volume of 3.5M NaOH and diluted with 500µL of 0.2M citrate buffer (pH 2.2). The hydrolysate was injected into an amino acid analyzer HP1100 (Agilent, USA) [17].

2.3.4 Denaturation temperature

The denaturation temperatures of the collagen extractswere determined using an Ostwald's viscometer [18]. Collagen solution (1%) in acetic acid was filled in the viscometer, immersed in a water bath at 30°C, and kept for 30 min to equilibrate the temperature of the collagen solution. The temperature was raised step by step to 50°C with an interval of 2°C and maintained for 10 min at each temperature. The specific viscosities of the samples were calculated followed by

measurement of the fractional viscosities [19]. The thermal denaturation curve was plotted between fractional viscosity and temperature. The denaturation temperature was taken as the temperature at which the fractional viscosity was 0.5.

2.3.5 Solubility – effect of pH and NaCl

The extracted collagen was made into 3 mg/mL concentration by mixing with 0.5M acetic acid and stirring well at 4℃ until all the collagenshad dissolved. Five ml of the above solution was plated in individual tubes and the pH was adjusted from 1 to 12 using HCl and NaOH. After making the total volume uniform using distilled water, all the tubes were incubated at 4℃ for 30 min. The contents were centrifuged after 12,000rpm for 30 min at 4℃ to remove the precipitated protein fractions. The same procedure was repeated with different concentrations of NaCl (0-12%) and concentrations of soluble protein in the supernatants were calculated. The concentration of soluble protein was measured in the supernatant and the solubility was calculated using the total protein concentration value [20].

2.3.6 Antimicrobial activity

The antimicrobial activity of the extracted collagen was predicted by the well diffusion method. *Escherichia coli, Pseudomonas* sp., *Staphylococcus* sp., and *Klebsiella* sp. were the selected pathogens for the antimicrobial study, and they were plated in four different sterilized agar plates in triplicates. Four wells were punctured in each plate and to each were added three different concentrations of collagen (25 µL, 50 µL, 100 µL) in three wells, and the solvent in the fourth well as a control. After the collagen solution of different concentrations diffused into the agar, the plates were incubated at 37℃ for 24 h. The zone of clearance that appeared around each well was measured and tabulated.

2.4 *In vitro* study of wound healing

In vitro studies on the wound healing capacity of the collagen extracts were performed using animal cell lines. A549 cell line (Adeno carcinomic human alveolar basal epithelial cells) through explant culture of lung carcinomatous tissue was used for this study. The cells were cultured in F-12K medium along with fetal bovine serum to a final concentration of 10% with atmospheric air 95%, carbon dioxide 5%, and temperature 37℃. Subculturing was done by removing and replacing the culture medium gently without affecting the cell lines. Preservation of cultured cells was done by adding a freezing medium supplemented with 5% (v/v) DMSO and storing them in liquid nitrogen. The cell layers were rinsed with 0.25% (w/v) trypsin - 0.53 mM EDTA solution to remove any trypsin inhibitor that might be present in the serum. After rinsing, 3 mL of trypsin-EDTA solution was added to the cells and examined in a phase contrast microscope for cell dispersion. The culture flasks were kept at 37℃ for efficient dispersion and were not disturbed or shaken as thiscould have led to the clumping of cells and improper dispersion. Then 8-10 mL of culture medium was added to the flasks and appropriate aliquots of the cell suspensions were transferred to new flasks and incubated at 37℃. The wound healing assay was performed based on the ability of collagen to enhance the regeneration capacity of the dispersed cells. Fifty µL of extracted collagen (1mg/mL) was coated in 12 well plates as triplicate for 24 h. After coating, the cells were seeded in the plates and incubated. Cells which were seeded with cells but not coated with collagen were used as controls.

3. Results and Discussion

3.1. Collagen extraction

Acid-soluble collagen (ASC) was extracted from the fish skin and scales of the selected fish species. Among the three species, *N. ductor* showed a maximum extraction of collagen both from the skin (51.55±1.02%) and scale (41.02±0.02%) compared to the other two species. The least concentration of collagen extract was reported in *C. carpio*, 32.03±0.25%, and 15.11±0.39% from skin and scales, respectively (Figure 1). The Duncan Multiple Range Test (DMRT) revealed a significant difference of collagen in the skin and scales of *C. carpio, O. mossambicus*, and *N. ductor* at $P < 0.05$ The yield of ASC from Brownbanded bamboo shark (*Chiloscyllium punctatum*) was 9.38% (wet weight basis) [21], and the yield from bigeye snapper skin was 6.4% (wet weight basis), whereas for brownstripe red snapper skin, a yield of ASC was 9% (wet weight basis) was reported [22].

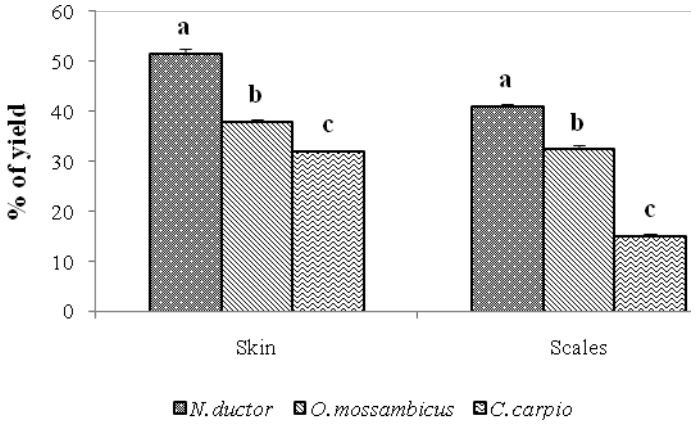

Figure 1. Concentration of collagen from skin and scales of *N. ductor*, *O. mossambicus*, and *C. carpio*.
Experiments were performed in triplicate and the values are representing as mean±SD. Different superscripts on the above columns indicated significant differencesbetween the species at $P < 0.05$.

3.2. Characterization of collagen

3.2.1 Total protein

Naucrates ductor and *O. mossambicus* showed significant protein concentrations in the collagen extract. The total protein contents were measured by Lowry's method and maximum protein content (160.67±1.15 mg/mL) was reported in the skin of *N. ductor* and the minimum (55.33±0.58 mg/mL) in the scales of *C. carpio*. Among the three species, the protein content of *N. doctor* and *O. mossambicus* were found to be similar but *C. carpio* exhibitedmuch lower values. This might have been due to the variations in the protein metabolic pathways of different fishes. The DMRT revealed a significant difference of protein in the skin and scales of *C. carpio, O. mossambicus*, and *N. ductor* at $P < 0.05$ (Figure 2). Similar results for the protein content were observed in the skin and bones of rainbow trout [23] and tuna, dog shark and rohu [24].

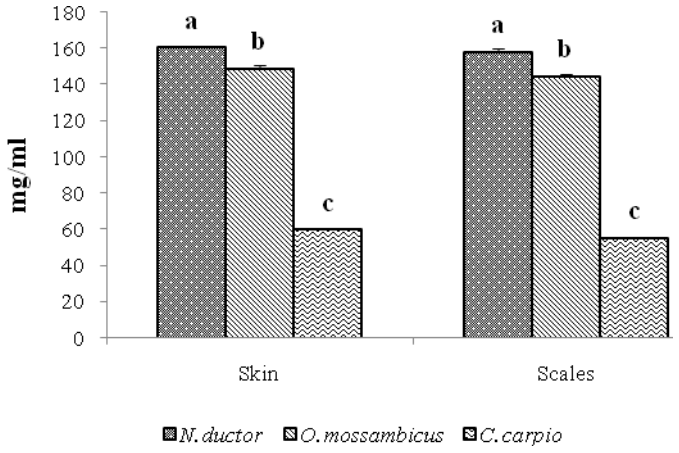

Figure 2. Total protein concentration in collagen extracted from skin and scales of *N. ductor*, *O. mossambicus*, and *C. carpio*.
Experiments were performed in triplicate, and the values represent mean±SD. Different superscripts on the above columns showed significant differencesbetween the species at $P < 0.05$.

3.2.2 Molecular weight and subunit composition

Molecular weight analysis of extracted collagen was performed in SDS-PAGE using 7.5% gels. Molecular weight marker protein was loaded in lane 1. Lanes 2, 3, and 4 were loaded with the collagen extracted from the skin of *N. ductor*, *O. mossambicus*, and *C. carpio*, respectively. Similarly, lanes 5, 6 and 7 were loaded with the collagen extracted from the scales of *N. ductor*, *O. mossambicus*, and *C. carpio*, respectively. The SDS-PAGE pattern showed that all collagens had a doublet pattern for α1 and α2 chains (approximately between 97.4kDa and 116kDa position) and a β chain (approximately near to 185kDa position). The band intensity was measured using a gel documentation system and the intensity of the α1 subunit was approximately twice that of the α2 in all samples, indicating the presence of more α1 chains in the collagen structure. The presence of bands in lanes 2,4 and 7 above the 205kDA marker indicated that it contained a heavy γ chain with a molecular weight of around 210kDa (Figure 3). The patterns of collagens from the skin and scales of fish were similar to the standard acid-soluble type 1 calf-skin collagen. Similar results were observed in deep-sea redfish [25], Nile perch [26], and brownstripe red snapper [22].

3.2.3 Amino acid profiling

The amino acid compositional analysis showed that about one-third of the total amino acid content was glycine, which is a unique characteristic of animal collagen. A higher number of alanine, proline, hydroxyproline, and glutamine were reported in the present study (Table 1). On the other hand, the extracted fish collagen had a lower content of cysteine, histidine, and hydroxylysine and no tryptophan. The amino acid profiles reported here were consistent and were in agreement with the amino acid profiles of collagens reported in earlier literature [27-29].

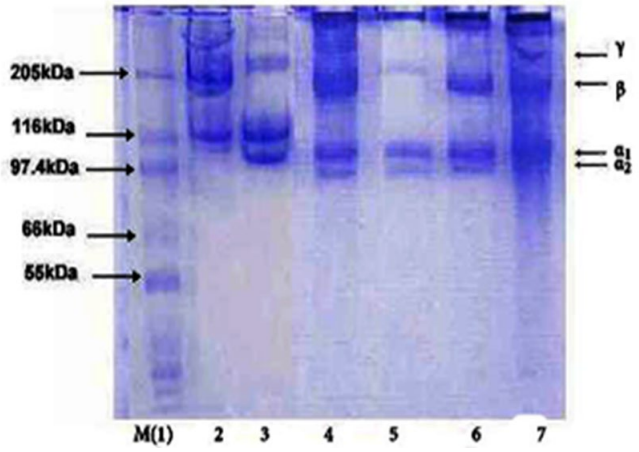

Figure 3. SDS-PAGE pattern of collagen extracted from different parts of the fish (skin and scales) on 7.5% gels. Lane 1, molecular weight marker(M); lanes 2, 3 and 4 show the skin collagen of *N. ductor*, *O. mossambicus*, and *C. carpio*; lanes 5, 6 and 7 show the scale collagen of *N. ductor*, *O. mossambicus*, and *C. carpio*.

Table 1. Amino acid analysis of collagen extracted from skin and scales of different species of fish

	Skin			**Scales**		
	N. ductor	***O. mossambicus***	***C. carpio***	***N. ductor***	***O. mossambicus***	***C. carpio***
Alanine	112	109	101	110	107	101
Arginine	55	51	48	55	52	48
Aspargine	46	42	40	47	42	40
Cysteine	2	2	1	1	1	1
Glumatine	81	78	74	81	78	74
Glycine	341	332	258	338	332	237
Histidine	8	8	7	8	8	7
Isoleucine	22	20	18	22	21	18
Leucine	27	23	20	25	23	20
Hydroxylysine	9	7	6	8	7	6
Hydroxy proline	96	88	86	93	87	81
Proline	104	99	80	99	99	70

3.2.4 Denaturation temperature

Denaturation temperatures were calculated by measuring the fractional viscosities of the collagen at different temperatures. Denaturation temperatures were predicted for the collagen extracted from the skin of *N. ductor*, *O. mossambicus*, and *C. carpio* as 41.83±0.29°C, 41.00±0.87°C and 38.33±0.29°C, respectively. For the collagen extracted from scales of *N. ductor*, *O. mossambicus*, and *C. carpio*, the denaturation temperatures were predicted as 42.33±0.29°C, 40.50±0.50°C, and 37.33±0.29, respectively (Figure 4). Compared to mammalian sources, the denaturation temperature of fish skin, scales, and fins were slightly lower [30] except for fish bone collagen. Generally, the denaturation temperature of fish collagen in cold environments is lower than in warm climates, because collagen from fish in cold environments has lower hydroxyproline content [31]. The imino rings present in proline and hydroxy proline hindered rotational movement of the C-N backbone of amino acids, conferring confirmational stability and higher denaturation temperature [32].

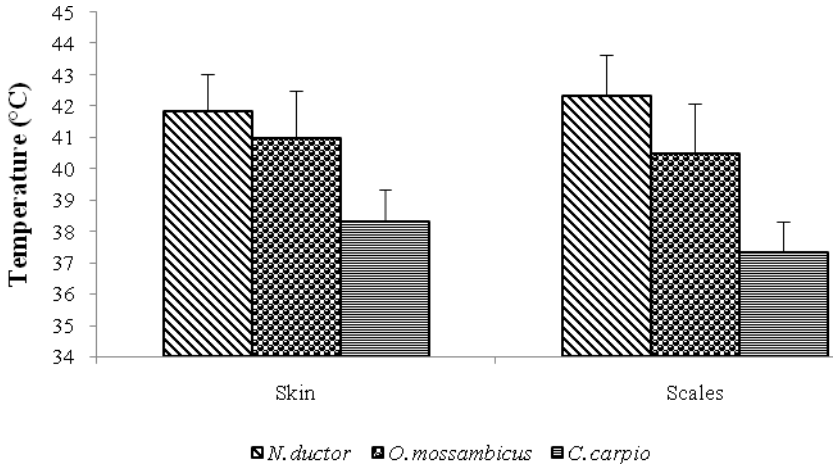

Figure 4. Denaturation temperature of the collagen extracted from the skin and scales of *N. ductor*, *O. mossambicus*, and *C. carpio*

3.2.5 Solubility – effect of pH and NaCl

The solubility of collagen extracts was found to be maximum under highly acidic conditions and started to decrease as the acidity decreased. The lowest solubility values were reported in the pH range of 5 to 7, and slowly started to increase again as the alkalinity increased (Figure 5). Generally, both skin and scale collagens could be more soluble at acidic pH [33]. At very acidic pH, skin collagen had a higher solubility than scale collagen. Acid-soluble collagen from the skin and bone of rainbow trout had the maximum solubility at pH 1 to 4 and the minimum solubility at pH 9 and 7 [34].

NaCl concentration showed a negative effect on solubility. With increasing NaCl concentration, the solubility decreased linearly for collagens extracted from both the skin and scales (Figure 6). There was no significant change in the solubility of collagen after increasing the concentration of NaCl beyond 8% as the maximum amount of proteins had been precipitated and saturation was reached. Similar studies were done and the solubility of the collagen from the skin and bones of rainbow trout was found to decrease due to the increase in salt concentration [34].

3.2.6 Antimicrobial activity

The antimicrobial activity of the collagen extracted from the skin and scales of the three fish varieties was measured by the agar well-diffusion method for the following bacterial pathogens: *E. coli, Pseudomonas* sp., *Staphylococcus* sp., and *Klebsiella* sp. Three different dosage volumes (25 µL, 50 µL, and 100 µL) were used in this study. The inhibition radius grew with increase in dosage volume of collagen irrespective of the fish pathogens. The zone of inhibition radius varied with the bacterial species and source of collagen. When comparing the results of fish skin and scale-based collagen, skin-derived collagen exhibited better antibacterial properties than scale-derived collagen against all four selected pathogens with varying dosage volumes. The skin-derived collagen of *N. ductor* displayed maximum antibacterial properties against *Klebsiella* sp. and *Pseudomonas* sp. while the skin-derived collagen of *C. carpio* was effective against *E. coli* and *Staphylococcus* sp. (Tables 2 and 3). The collagen gelatin extracted from the skin of squid and tuna against *L. acidophilus*, *B. lactis*, *S. putrafaciens*, and *P. phosphoreum* shows better

antimicrobial activity and fractions of low molecular weight range of 1-10 kDa and <1 kDa from tuna seemed to be more active than extracts from squid and the results showed the antibacterial activity of the fractions was found both on Gram-positive and Gram-negative bacteria [35]. Collagen peptides were used in this investigation for their antibacterial properties, and they demonstrated full spectrum antibacterial activity against all bacterial strains. Some of these interacting antimicrobial peptide mechanisms have already been reviewed [36].

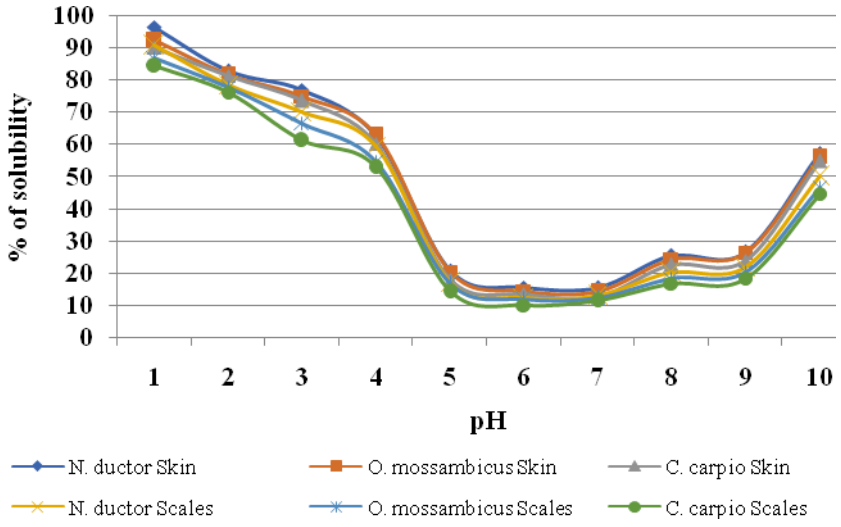

Figure 5. Effect of pH on the solubility of collagen extracted from skin and scales

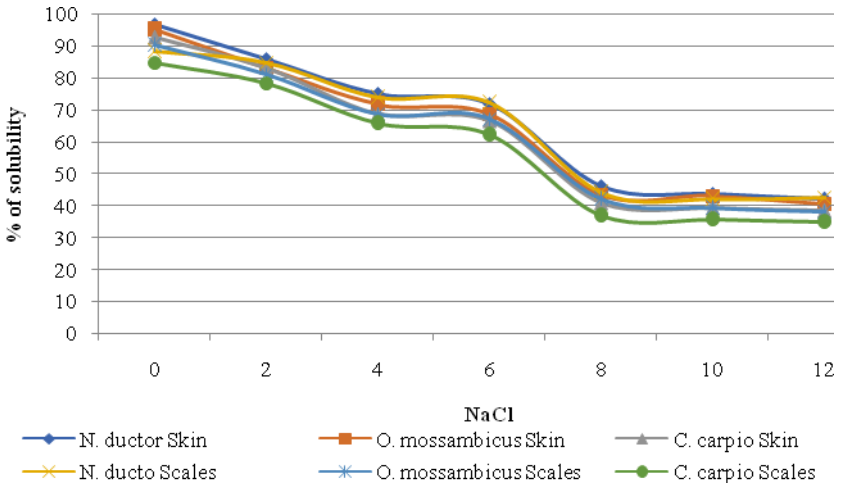

Figure 6. Effect of NaCl concentration on the solubility of collagen extracted from skin and scales

Table 2. Antimicrobial activity of collagen extracted from skin

Concentration	Fishes	*E. coli*	*Pseudomonas* sp.	*Staphylococcus* sp.	*Klebsiella* sp.
25 µL	*N. ductor*	11.20±0.10[a]	11.40±0.26[a]	11.80±0.26[a]	11.73±0.27[a]
	O. mossambicus	10.68±0.20[a]	11.20±0.26[a]	11.73±0.21[a]	11.50±0.20[a]
	C. carpio	10.47±0.25[a]	9.57±0.21[b]	10.53±0.25[b]	11.47±0.25[a]
50 µL	*N. ductor*	12.81±0.60[a]	15.21±0.08[a]	13.53±0.25[a]	15.70±0.20[a]
	O. mossambicus	12.77±0.25[a]	14.67±0.15[b]	13.77±0.25[a]	14.37±0.15[b]
	C. carpio	12.90±0.10[a]	12.81±0.24[c]	12.73±0.21[b]	14.16±0.35[b]
100 µL	*N. ductor*	16.51±0.17[a]	17.50±0.30[a]	15.73±0.25[a]	18.16±0.29[a]
	O. mossambicus	15.17±0.15[b]	16.63±0.15[b]	15.57±0.31[a]	16.47±0.21[b]
	C. carpio	17.03±0.15[c]	15.07±0.21[c]	16.20±0.20[b]	15.20±0.27[c]

Mean±SD of triplicate values
Anova followed by DMRT test performed at $P < 0.05$
The fishes of particular concentrations were distinguished by various superscripts in the same column, which revealed a significant difference ($P < 0.05$).

Table 3. Antimicrobial activity of collagen extracted from scales

Concentration	Fishes	*E. coli*	*Pseudomonas sp.*	*Staphylococcus sp.*	*Klebsiella sp.*
25 µL	*N. ductor*	9.27±0.25[a]	9.90±0.10[a]	10.11±0.13[a]	11.20±0.26[a]
	O. mossambicus	8.57±0.31[b]	10.13±0.32[b]	10.40±0.30[a]	10.70±0.20[b]
	C. carpio	9.33±0.35[a]	9.00±0.10[a]	9.53±0.25[b]	10.43±0.25[b]
50 µL	*N. ductor*	11.27±0.25[a]	14.30±0.30[a]	12.27±0.25[a]	14.13±0.15[a]
	O. mossambicus	10.30±0.20[b]	13.70±0.20[b]	13.00±0.10[b]	13.73±0.21[b]
	C. carpio	10.30±0.20[b]	12.27±0.18[c]	11.33±0.15[c]	13.03±0.15[b]
100 µL	*N. ductor*	14.50±0.20[a]	16.50±0.20[a]	15.50±0.20[a]	16.60±0.10[a]
	O. mossambicus	14.60±0.26[a]	15.33±0.15[b]	14.27±0.25[b]	15.77±0.25[b]
	C. carpio	14.40±0.56[a]	14.73±0.21[c]	11.03±0.58[c]	14.40±0.40[c]

Mean±SD of triplicate values
Anova followed by DMRT test performed at $P < 0.05$
The fishes of particular concentrations were distinguished by various superscripts in the same column, which revealed a significant difference ($P < 0.05$).

3.3 *In vitro* wound healing studies

Trypsin-EDTA-mediated cell dispersion was initiated in A549 cell lines (Figure 7a) for the wound healing assay and examined by phase contrast microscopy. Cell lines were added with collagen extracted from the three fish species used in this study. Dispersed cell lines without collagen extract were used as the control (Figure 7b) for examining the wound healing capacity of the collagen extracts (Figures 7c-f). After 24 h of incubation, the migratory and proliferative nature of cells was observed in the cell lines (Figures 7g-j). Almost 95% of healing was observed in 48 h in the collagen-treated cell line whereas cell proliferation of that magnitude was not observed in the

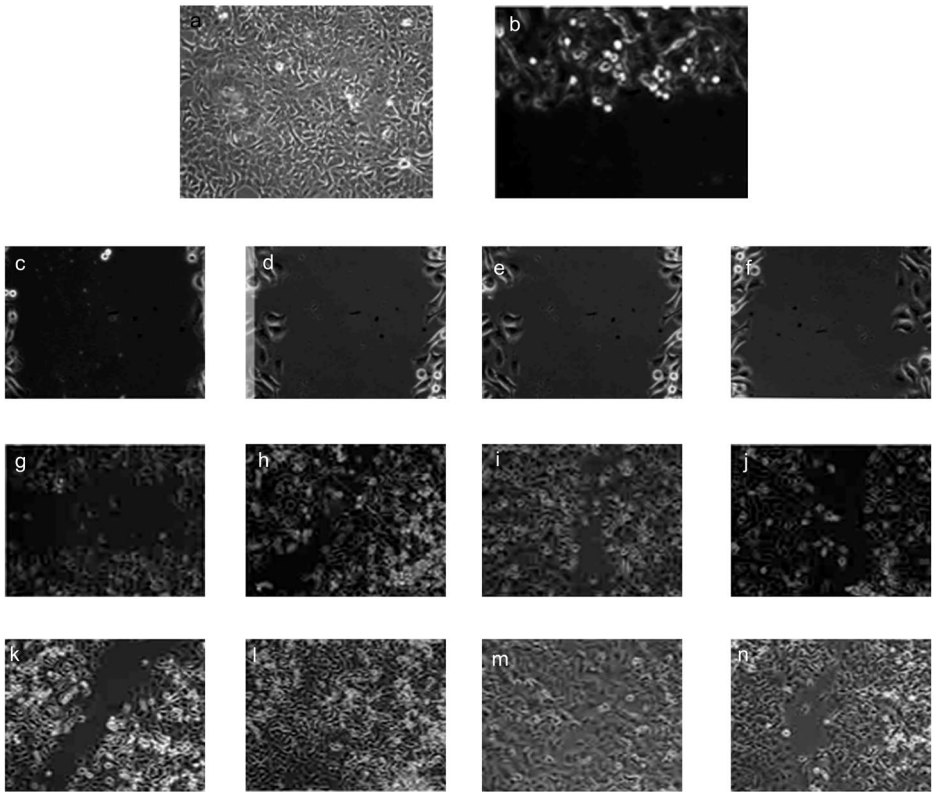

Figure 7. a. A549 Cell line (Normal Cell Line); **b.** A549 Cell line (Wound Cell line); **c.** Wound healing without collagen (control); **d.** Wound healing with *N. ductor* Collagen; **e.** Wound healing with *O. mossambicus* Collagen; **f.** Wound healing with *C. carpio* Collagen; **g** Control cell line after 24 h; **h** *N. ductor* collagen treated wound cell line after 24 h; **i** *O. mossambicus* collagen treated wound cell line after 24 h; **j** *C. carpio* collagen treated wound cell line after 24 h; **k** Control cell line after 48 h; **l** *N. ductor* collagen treated wound cell line after 48 h; **m** *O. mossambicus* collagen treated wound cell line after 48 h; **n** *C. carpio* collagen treated wound cell line after 48 h

control (Figures 7k-l). The enhancement of cell migration and proliferation by the extracted fish collagen was the reason for this boosted wound repair phenomenon. Among these, collagen from *N. ductor* showed better cell regeneration capacity. Similar studies showed the proliferation and migration of 3T3 fibroblasts with poly-herbal formulation [37].

4. Conclusions

The research work focuses on the extraction of fish collagen which possesses so many useful traits that makes it useful in the healthcare sector. Three fish species were selected, and collagen extraction was performed from their skin and scales which are often dumped as waste. Amaximum percentageextractionof 51.55% was reported in the skin of *N. ductor* among the selected fish species. The extracted collagen was characterized based on its total protein content, molecular weight of protein subunit, denaturation temperature, and solubility at different ranges of pH and salinity. Maximum protein content was reported in the skin of *N. ductor* and minimum in the

scales of *C. carpio*. For both skin and scale-derived collagen, only small differences in the denaturation temperature were found between the three species among which *N. doctor* shows maximum denaturation temperature followed by *O. mossambicus*, and *C. carpio*. Solubility values of both skin and scale-derived collagen followed the same pattern and were found to be lowest at the pH range between 5 to 7. Amino acid analysis of the extracted collagen indicated a high fraction of glycine, which is a unique characteristic of collagen. The antimicrobial activity of the collagen extracts was measured against four bacterial pathogens and gave promising results. The wound-healing nature of collagen was examined in A549 cell lines, and 95% of cell regeneration and migration was reported.

5. Acknowledgments

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