Media Kesehatan Politeknik Kesehatan Makassar
ISSN : 1907-8153 (Print)
e-ISSN : 2549-0567 (Online)

Formulation and Anti-Aging Activity of Collagen Extract from Yellowfin Tuna (Thunnus albacares) Skin Cream in UV-B Induced Wistar Rats

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Article Information: Received February 2026; Accepted June 2026; Published June 2026

ABSTRACT

Skin aging is a progressive biological process that can be accelerated by excessive ultraviolet (UV) exposure, leading to structural damage characterized by wrinkle formation, loss of elasticity, and rough skin texture. This study aimed to formulate an anti-aging cream containing collagen-rich skin extract of yellowfin tuna (*Thunnus albacares*) and to evaluate its anti-aging efficacy in a UV-B–induced skin aging model using Wistar rats (*Rattus norvegicus*). A laboratory experimental design was employed using 15 male rats divided into five groups: negative control (cream base), positive control (commercial anti-aging cream), and treatment groups containing 1%, 3%, and 5% yellowfin tuna skin extract. The dorsal skin was exposed to UV-B radiation for 17 minutes daily for 10 consecutive days, followed by topical application of the respective formulations. Wrinkle severity was assessed macroscopically using a visual scoring system (0 = normal texture; 1 = shallow wrinkles; 2 = deep wrinkles with laxity). All formulations demonstrated acceptable physical stability, with pH values ranging from 4.98 to 5.48 and viscosity remaining within the acceptable range after cycling tests. Anti-aging evaluation revealed a dose-dependent improvement in wrinkle scores. The 5% formulation showed the most pronounced effect, with the median wrinkle score decreasing from 2 on day 1 to 0 on day 10, whereas the negative control group showed only a slight reduction from 2 to 1 during the same period. The 3% formulation demonstrated moderate improvement, while the 1% formulation exhibited fluctuating results. In conclusion, yellowfin tuna skin extract can be successfully formulated into a stable oil-in-water cream, and the 5% concentration exhibited the highest anti-aging activity in UV-B–exposed rats, supporting its potential as a marine-derived natural ingredient for topical anti-aging applications.

Keywords: *Thunnus albacares*; Collagen; Skin Aging; Anti-Aging Agents; Ultraviolet Rays; Rats, Wistar

INTRODUCTION

Skin aging is a degenerative process caused by a decline in physiological functions. Part of the aging process is attributed to intrinsic factors; however, extrinsic factors such as sun exposure, pollution, and lifestyle significantly increase oxidative stress and contribute to chronic inflammation, thereby accelerating skin aging. Ultraviolet (UV) radiation, including UVA and UVB, plays a major role in daily skin damage. Studies have demonstrated that prolonged exposure of human skin to external stimuli such as UV radiation induces damage to cell viability, membrane integrity, and elastic tissue. This damage disrupts the structure of the skin's extracellular matrix, leading to clinical manifestations such as wrinkle formation and even skin cancer [(1, 2)].

Collagen is one of the most abundantly produced proteins in the human body. The major types of collagen include type I collagen, which is found in the skin, tendons, and bone tissue; type II collagen, which is predominantly present in cartilage; and type III collagen, which is found in the skin and blood vessels [3]. Collagen is responsible for maintaining tissue strength by forming a supportive network along cellular structures. However, with increasing age, collagen fibers gradually degrade, resulting in undesirable effects such as wrinkle formation on the skin [4].

The utilization of animal meat and skin in wound management is based on their ability to facilitate the wound-healing process. The primary biomaterial involved is collagen, which is abundantly present in fish skin and can be identified by its elastic and resilient texture. In addition to its role in wound healing, collagen is also capable of restoring skin function damaged by aging. Collagen exhibits antioxidant properties that effectively counteract the accumulation of free radicals in the skin, making it a promising anti-aging agent against oxidative stress induced by UV exposure [5]. The proportion of tuna skin can account for approximately 5–8% of the total body weight of tuna. The proximate composition of yellowfin tuna skin consists of 60.22% moisture, 36.09% protein, 2.25% ash, and 1.08% fat. These characteristics indicate that yellowfin tuna skin has significant potential as a raw material for collagen production [(6, 7)].

Collagen is commonly derived from terrestrial animals such as cattle and pigs. However, disease outbreaks including bovine spongiform encephalopathy (BSE), transmissible spongiform encephalopathy (TSE), and foot-and-mouth disease (FMD), as well as religious restrictions particularly among Muslim communities have raised concerns regarding the use of collagen from these sources. Consequently, alternative collagen

ISSN : 1907-8153 (Print)
e-ISSN : 2549-0567 (Online)

sources are required, one of which is fish-derived collagen obtained from fish skin, fins, scales, and bones. Collagen is widely utilized in the fields of biomaterials, food, pharmaceuticals, and cosmetics due to its excellent biocompatibility and biodegradability (8, 9).

A study conducted by Nurjanah reported that the molecular weights of papain-soluble collagen extracted from yellowfin tuna skin were β (310 kDa), α1 (150 kDa), and α2 (113 kDa) [10]. Meanwhile, the molecular weights of pepsin-soluble collagen from yellowfin tuna skin were β (328 kDa) and α1 (171 kDa) [11]. Collagen content in the skin decreases with age, which has driven intense competition within the cosmetic industry to develop collagen-based products for both topical and oral applications. In cosmetic formulations, collagen is used as an active ingredient in skincare products to enhance skin hydration, prevent wrinkle formation, protect the skin from harmful radiation, and maintain skin elasticity. Due to its high biocompatibility, non-toxicity, and biodegradability, collagen exhibits a broad spectrum of applications across the cosmetic, pharmaceutical, medical, and food industries [4].

The incorporation of collagen into cosmetic formulations is intended to replace collagen that has been damaged due to environmental exposure or aging-related factors. The advantages of collagen utilization are associated with its physicochemical characteristics, including high absorption capacity in the body, low antigenicity, strong water-binding affinity, non-toxicity, biocompatibility, biodegradability, relative stability, and ease of dissolution in water or acidic solutions [12]. Collagen penetrates the skin layers and continuously forms a colloidal system on the skin surface, resulting in a smooth and soft skin sensation. Considering these properties, collagen is highly suitable for application as an anti-aging cosmeceutical ingredient [(13, 14)].

Cream formulations, particularly those intended for anti-aging applications, are widely used due to several advantages, including ease of application, greater comfort for facial use, and ease of removal with water [15]. The benefits of anti-aging cream application include the reduction of spots, wrinkle lines, and skin discoloration resulting from hyperpigmentation [(14, 15)]. Collagen derived from fish skin has a strong water-binding capacity, which helps maintain skin hydration and improve skin elasticity. In cream formulations, collagen performs effectively because the thicker texture of creams allows for more intensive moisture retention on the skin [14].

Fish bone collagen from Pangasius sp. has been formulated into anti-aging creams at collagen concentrations of 1%, 1.5%, 2.5%, and 3.5% [(17, 18)]. The incorporation of collagen in such formulations serves as an active ingredient that contributes to improvements in skin texture, hydration (moisture content), wrinkles, spots, and pore appearance. In addition, tuna skin collagen has been utilized in the formulation of anti-aging serum preparations. Collagen serum derived from tuna skin has been reported to prevent photoaging by providing up to 50% protection in UVB-exposed rats [(19, 20)]. In this study, the term "yellowfin tuna skin extract" specifically refers to acid-soluble collagen obtained through alkaline pretreatment and acetic acid extraction, followed by freeze-drying. Thus, the active ingredient evaluated in this formulation is collagen derived from yellowfin tuna skin rather than a crude extract containing multiple bioactive compounds.

MATERIALS AND METHODS

Study Design

This study employed an experimental research design aimed at formulating and evaluating the anti-aging activity of a cream preparation containing yellowfin tuna (Thunnus albacares) skin extract in Wistar rats (Rattus norvegicus). A total of 15 rats were used in this study and divided into five experimental groups. This research protocol received ethical approval from the Animal Research Ethics Committee of the Health Polytechnic of the Indonesian Ministry of Health, Makassar, with approval number 1214/M/KEPK-PTKMS/VII/2024.

Study Location

The study was conducted at the Pharmaceutical Dosage Form Technology Laboratory, the Pharmacology Laboratory of Megarezky University, Makassar, and the Chemistry Laboratory of the Ujung Pandang State Polytechnic. The research was carried out from June to December 2025.

Instruments and Materials

The instruments used in this study included a glass stirring rod (Iwaki), 500 mL beaker glass (Pyrex), porcelain crucible (Pyrex), glass funnel (Pyrex), deg glass, freeze dryer, 50 mL graduated cylinder (Pyrex), scissors, hot plate (Maspion), watch glass, animal cages, gauze, parchment paper, pH paper, pH meter (Phep), mortar and pestle (OneMad), UV-B lamp, microscope slides, water bath (Haru Tech), dropper pipette, horn spoon, spatula, analytical balance (Ohaus), and containers. The materials used in this study were yellowfin tuna skin, stearic acid, acetic acid, alcohol, distilled water (aquadest), butylated hydroxytoluene (BHT), methyl paraben, methylene blue, sodium hydroxide, oleum rosae, propylene glycol, propyl paraben, cetyl alcohol, and triethanolamine (TEA).

ISSN : 1907-8153 (Print)
e-ISSN : 2549-0567 (Online)

Experimental Procedures

Sample Collection and Preparation

Yellowfin tuna (Thunnus albacares) skin samples were obtained from East Kalimantan, Sangga Utara District, East Kutai Regency. The collected tuna skin was cut into small pieces and washed thoroughly under running water to remove impurities and residual adhering flesh [21, 7].

Sample Extraction

The extraction of collagen from yellowfin tuna (Thunnus albacares) skin was conducted following the method described by on tuna skin deproteinization pretreatment [22]. The deproteinization process aimed to remove non-collagenous proteins and lipids using sodium hydroxide (NaOH). Pretreatment of the yellowfin tuna skin was carried out by immersing the samples in a 0.1 M NaOH solution at a sample-to-solution ratio of 1:10 (w/v) for up to 24 hours. The samples were then washed thoroughly with distilled water until a neutral pH was achieved [23].

Collagen Extraction: Acid-soluble collagen extraction was performed by soaking the deproteinized samples in 0.5 M acetic acid at a ratio of 1:10 (w/v) for 72 hours (3 days)[4]. The extended soaking duration facilitated increased water absorption by the skin tissue, thereby enhancing collagen separation and improving extraction efficiency [9].

Following acid extraction, the skin samples were neutralized using distilled water until neutral pH was reached. The samples were subsequently extracted with distilled water at 45 °C for 120 minutes, using a sample-to-solvent ratio of 1:2 (w/v). The resulting extract was obtained in liquid collagen form and subsequently dried using a freeze dryer to produce dried collagen powder.

Formulation of Anti-Aging Cream Containing Yellowfin Tuna Skin Extract

After extraction, the collagen sample was formulated into an anti-aging cream preparation at different concentrations. The composition of the anti-aging cream formulations is presented in Table 1.

Table 1. Formulation of anti-aging cream containing yellowfin tuna (Thunnus albacares) skin collagen extract.

Ingredient	Formula (%) (w/v)					Function
	F1(1%)	F2(3%)	F3(5%)	C-	C+	
Yellowfin tuna skin collagen extract	1	3	5	0	Wardah anti-aging cream	Active ingredient
Stearic acid	5	5	5	5		Emulsifier
Cetyl alcohol	5	5	5	5		Emollient
Propylene glycol	3	3	3	3		Humectant
Methyl paraben	0,2	0,2	0,2	0,2		Preservative
Propyl paraben	0,5	0,5	0,5	0,5		Preservative
Triethanolamine (TEA)	1	1	1	1		Emulsifier
Butylated hydroxytoluene (BHT)	0,1	0,1	0,1	0,1		Antioxidant
Rose oil	qs	qs	qs	qs		Fragrance
Distilled water	Ad 30	Ad 30	Ad 30	Ad 30		Vehicle/Solvent

The preparation of the anti-aging cream was divided into two phases: an aqueous phase and an oil phase. The aqueous phase consisted of propylene glycol, triethanolamine, and methyl paraben (nipagin), which were dissolved in a measured volume of hot distilled water (23, 24). The oil phase comprised stearic acid, cetyl alcohol, butylated hydroxytoluene (BHT), and propyl paraben, which were melted using a water bath. The oil phase was then gradually added to the aqueous phase in a mortar and mixed continuously until a homogeneous cream base was formed. The collagen extract was added gradually while stirring until a uniform and homogeneous consistency was achieved [26]. Finally, five drops of fragrance were incorporated into the formulation. The same procedure was applied to all formulations, with collagen concentrations of 1%, 3%, and 5% according to the predetermined variations.

Evaluation of Cream Preparations

The evaluation of the anti-aging cream containing yellowfin tuna skin collagen extract included organoleptic testing, homogeneity assessment, pH measurement, viscosity determination, spreadability testing, and cream type identification.

ISSN : 1907-8153 (Print)
e-ISSN : 2549-0567 (Online)

Anti-Aging Activity Evaluation of Yellowfin Tuna Skin Extract Cream

A total of 15 male Wistar rats weighing approximately ±200 g were used as experimental animals. Prior to treatment, the animals were acclimatized to the laboratory environment for seven days to allow adaptation to the new conditions and experimental procedures. The rats were housed in cages and provided with sufficient food and water daily [27].

The rats were divided into five groups, each consisting of three animals. After acclimatization, the rats were labeled on their tails (I, II, and III) according to their respective treatment groups: 1%, 3%, and 5% extract concentrations, negative control, and positive control. The dorsal hair of each rat was removed by shaving an area of 5 × 5 cm. The shaved dorsal skin was then exposed to UV-B radiation using a UV-B lamp positioned approximately 10 cm above the skin surface. Irradiation was conducted for five days per week over a period of two weeks, with an exposure duration of 17 minutes per day [28].

To ensure adequate and uniform UV-B exposure, a restraint device was used during irradiation. The cream formulations were applied immediately after UV-B exposure and allowed to remain in contact with the dorsal skin for approximately five minutes. Group 1 received the cream formulation containing 1% extract, Group 2 received the 3% extract formulation, and Group 3 received the 5% extract formulation. Group 4 received the cream base as the negative control, while Group 5 received a commercial anti-aging cream, Wardah Renew You Anti-Aging Day Cream, as the positive control.

Wrinkle Observation After UV-B Exposure

Wrinkle observation following UV-B exposure was performed at the end of the second week using visual assessment based on a scoring system according to the observation criteria: 0 = normal skin texture; 1 = presence of shallow wrinkles; and 2 = presence of several deep wrinkles accompanied by skin laxity [27, 28].

Data Analysis

Data obtained from the evaluation of the cream preparations were statistically analyzed using the Statistical Package for the Social Sciences (SPSS). A paired sample t-test was employed to determine differences in the evaluation parameters of the cream formulations before and after the cycling test by assessing statistically significant changes. A p-value < 0.05 was considered indicative of a statistically significant difference, whereas a p-value > 0.05 indicated no statistically significant difference [29, 30].

RESULTS

1. **Yield of Yellowfin Tuna Skin Collagen Extraction (Thunnus albacares)**

The yield of collagen extracted from yellowfin tuna skin (Thunnus albacares) is presented in Table 2 :

Table 2. Yield of collagen extraction

Sample	Solvent Type	Sample Weight (g)	Extract Weight (g)	Yield (%)
Yellowfin tuna skin (*Thunnus albacares*)	0.1 M NaOH, 0.5 M acetic acid, and distilled water	200 g	4,90 g	2,45 %

2. **Evaluation of Anti-Aging Cream Containing Yellowfin Tuna Skin Extract**
Organoleptic Test

Table 3. Organoleptic test results

Formula	Texture		Color		Odor	
	Before Cycling test	After Cycling test	Before Cycling test	After Cycling test	Before Cycling test	After Cycling test
C-	Semi-solid	Semi-solid	White	White	Characteristic	Characteristic
F1(1%)	Semi-solid	Semi-solid	Pale white	Pale white	Characteristic aromatic	Characteristic aromatic
F2(3%)	Semi-solid	Semi-solid	Slightly darker white	Slightly darker	Characteristic aromatic	Characteristic aromatic
F3(5%)	Semi-solid	Semi-solid	Darker white	Darker	Characteristic aromatic	Characteristic aromatic

ISSN : 1907-8153 (Print)
e-ISSN : 2549-0567 (Online)

Homogeneity Test

Table 4. Homogeneity test results

Formula	Observation		Requirement
	Before Cycling test	After Cycling test	
C-	Homogeneous	Homogeneous	Homogeneous [32].
F1(1%)	Homogeneous	Homogeneous	
F2(3%)	Homogeneous	Homogeneous	
F3(5%)	Homogeneous	Homogeneous	

pH Test

Table 5. pH test results

Formula	Observation		Requirement	Remarks
	Before Cycling test	After Cycling test		
C-	5,01 ± 0,10	5,03 ± 0,12	4,5 – 6,5 [16].	0,066 > 0,05
F1(1%)	4,98 ± 0,11	5,01 ± 0,17		
F2(3%)	5,16 ± 0,32	5,27 ± 0,22		
F3(5%)	5, 39 ± 0,22	5,48 ± 0,11		

Spreadability Test

Table 6. Spreadability test results

Formula	Spreadability (Mean±SD)		Requirement	Remarks
	Before Cycling test	After Cycling test		
C-	5,99 ± 0,12	6,00 ± 0,15	5-7 cm [33].	0,051 > 0,05
F1(1%)	5,68 ± 0,02	5,71 ± 0,14		
F2(3%)	5,47 ± 0,13	5,53 ± 0,02		
F3(5%)	5,23 ± 0.12	5,26 ± 0,04		

Viscosity Test

Table 7. Viscosity test results

Formula	Observation (Mean±SD)		Requirement	Remarks
	Before Cycling test	After Cycling test		
C-	4500 ± 0,22	4380 ± 0,10	2000-50000 [34].	0,063 > 0,05
F1(1%)	5299 ± 0,32	5200 ± 0,25		
F2(3%)	8180 ± 0,34	7780 ± 0,32		
F3(5%)	12839 ± 0,25	12380 ± 0,27		

Cream Type Test

Table 8. Cream type identification

Formula	Observation		Remarks
	Before Cycling test	After Cycling test	
C-	O/W (M/A)	O/W (M/A)	Even distribution (O/W); blue spots indicate W/O
F1(1%)	O/W (M/A)	O/W (M/A)	
F2(3%)	O/W (M/A)	O/W (M/A)	
F3(5%)	O/W (M/A)	O/W (M/A)	

3. **Anti-Aging Activity Test in Wistar Rats**

Table 9. Anti-aging activity of yellowfin tuna skin extract cream in Wistar rats based on wrinkle score observations

Formula	Treatment	Wrinkle Observation Score on Day-									
		1	2	3	4	5	6	7	8	9	10
C+	Rat I	1	2	2	1	1	1	1	1	0	0
	Rat II	2	2	1	1	1	1	1	0	0	0
	Rat III	2	1	1	2	2	1	1	0	1	0

https://doi.org/ 10.32382/medkes.v21i1

Formula	Treatment	Wrinkle Observation Score on Day-									
		1	2	3	4	5	6	7	8	9	10
C-	Rat I	2	2	1	2	1	1	1	2	1	1
	Rat II	2	1	2	2	1	1	2	2	2	2
	Rat III	2	2	2	1	1	1	2	2	2	1
F1(1%)	Rat I	2	2	1	2	1	1	1	1	1	0
	Rat II	2	1	1	2	1	1	2	2	1	1
	Rat III	2	2	1	1	1	1	2	2	2	1
F2(3%)	Rat I	2	2	2	1	1	1	1	0	1	0
	Rat II	1	1	1	2	2	2	1	0	1	0
	Rat III	2	2	2	2	1	1	1	2	1	0
F3(5%)	Rat I	1	2	2	2	1	1	1	0	0	0
	Rat II	1	1	2	2	2	1	1	1	0	0
	Rat III	1	2	2	2	2	1	1	0	0	0

Wrinkle Scoring Criteria:
0 = normal skin texture
1 = presence of shallow wrinkles
2 = presence of several deep wrinkles accompanied by skin laxity

DISCUSSION

Collagen in cosmetic formulations is known to replace collagen that has been damaged due to environmental exposure or aging-related factors. The advantages of collagen utilization are closely related to its physicochemical characteristics, including high absorption capacity, low antigenicity, strong water-binding affinity, non-toxicity, biocompatibility, biodegradability, relative stability, and ease of dissolution in water or acidic solutions [(4, 34)]. Anti-aging creams are topical preparations designed to prevent or slow the aging process, thereby improving skin appearance and maintaining a youthful look [36]. In the present study, collagen extracted from yellowfin tuna skin was selected as the active ingredient in the anti-aging cream formulation [37]. The incorporation of yellowfin tuna skin collagen was expected to function as a moisturizing agent and to reduce wrinkle formation in the anti-aging cream preparation.

This study utilized yellowfin tuna (Thunnus albacares) skin as the raw material for collagen extraction. Yellowfin tuna is one of Indonesia's major export commodities. The proximate composition of yellowfin tuna skin consists of 60.22% moisture, 36.09% protein, 2.25% ash, and 1.08% fat. The high protein content of yellowfin tuna skin indicates its strong potential as a raw material for collagen production, which plays a crucial role in maintaining skin moisture and mitigating aging-related skin changes [(6, 37).] The initial stage of this study involved the deproteinization pretreatment of yellowfin tuna skin using a 0.1 M NaOH solution. Collagen, which is abundantly present in tuna skin, has a three-dimensional structure stabilized by peptide bonds. The collagen yield obtained in this study (2.45%) indicates that the extraction efficiency may have been influenced by pretreatment conditions. Compared with previous reports showing higher yields, this relatively lower recovery suggests that alkaline exposure duration or concentration may require further optimization. Excessive alkaline treatment can potentially reduce collagen integrity, thereby affecting final yield. Therefore, refining extraction parameters may improve recovery efficiency in future studies [39]. Compared with previous studies reporting yields of approximately 6.5%, the lower yield observed here may reflect differences in extraction protocols or biological variability of the raw material. Factors such as species variation, habitat conditions, and processing methods can significantly influence collagen recovery. Therefore, future studies should consider optimizing extraction parameters, including controlled alkaline exposure and potential enzymatic-assisted extraction methods.

Following the deproteinization process, the collagen extraction procedure proceeded to the hydrolysis stage, which involved the degradation of the material using 0.5 M acetic acid (CH_3COOH) for three days. The purpose of the hydrolysis process was to increase the concentration of H^+ ions, thereby facilitating water penetration into collagen fibers through electrostatic interactions with polar functional groups. This process induces structural changes in collagen proteins, particularly glycine-rich amino acid residues, which interact with acetic acid. These interactions affect the structural integrity of tropocollagen fibers, transforming them into procollagen through non-covalent bonding, ultimately enhancing collagen solubility during extraction [23]. The use of acetic acid in this study successfully facilitated collagen solubilization; however, the resulting yield suggests that acid concentration and extraction duration may influence recovery levels. Variability in raw material characteristics, such as fish age and post-harvest handling, may also contribute to differences in collagen extraction efficiency. These findings highlight the need for parameter optimization to maximize collagen recovery while preserving structural integrity[40].

After completion of the hydrolysis stage, the fish skin was extracted with water at 45 °C to prevent further denaturation and avoid the conversion of collagen into gelatin. The resulting liquid collagen extract was

subsequently dried using a freeze dryer to obtain dried collagen with a brownish appearance. Similar findings were reported by Dahliyanti and Shifani, who extracted collagen from selar fish skin (Selaroides leptolepis) and obtained brownish solid collagen [41].

The collagen extraction from yellowfin tuna skin yielded a collagen recovery rate of 2.45%. Collagen yield was calculated as the ratio of the dry collagen weight to the initial sample weight. Higher collagen yield corresponds to greater economic value. Paudi et al. reported a collagen yield of 1.84% from milkfish skin [42]. while Dewi et al. reported a yield of 6.5% from yellowfin tuna skin [5]. Another study reported a collagen yield of 41.5% from tilapia bone collagen [16]. Variations in collagen yield are influenced by differences in pretreatment methods, extraction techniques, and protein content of the samples used. In this study, the anti-aging cream formulations containing yellowfin tuna skin collagen were divided into four groups with different concentrations: F1 (1%), F2 (3%), F3 (5%), and C− (cream base without active ingredient). Each formulation underwent evaluation to ensure that the quality, safety, and functional benefits of the cream met the expected specifications and remained stable during storage. Stability assessments included organoleptic evaluation, homogeneity testing, pH measurement, spreadability testing, viscosity determination, and cream type identification.

The stability of the cream formulations was assessed using a cycling test conducted over six cycles. The cream samples were stored at a low temperature of 4°C for 24 hours and subsequently transferred to a high temperature of 40°C; this sequence was defined as one cycle. The physical characteristics of the cream formulations before and after the cycling test were then compared to evaluate stability. Organoleptic testing was performed to observe changes in color, texture, and odor of the yellowfin tuna skin extract cream through visual inspection before and after the cycling test. As shown in Table 3, prior to the cycling test, Formula 1 exhibited a pale white color, semi-solid consistency, and characteristic aromatic odor; Formula 2 showed a slightly darker white color, semi-solid consistency, and characteristic aromatic odor; and Formula 3 presented a darker white color, semi-solid consistency, and characteristic aromatic odor. The odor and color observed were attributed to the presence of yellowfin tuna skin extract. The negative control (cream base) displayed a white color, semi-solid consistency, and characteristic odor. Following the cycling test, the organoleptic properties of Formulas 1–3 and the negative control remained stable in terms of color, texture, and odor.

Homogeneity testing was conducted to evaluate whether the cream formulations were uniformly mixed. The test was performed by spreading a small amount of cream onto a microscope slide and covering it with another slide. Based on the results presented in Table 4, all formulations (Formulas I, II, III, and the negative control/base) demonstrated good homogeneity, as no coarse particles or lumps were observed on the slides for any formulation, both before and after the cycling test [43].

The pH (degree of acidity) of the cream formulations was measured using a pH meter. The pH meter probe was immersed into the cream sample, and the displayed pH value was recorded to determine whether the formulation was acidic or alkaline. An acidic pH may cause skin irritation, whereas an alkaline pH may result in dry and scaly skin. As shown in Table 5, all cream formulations exhibited pH values within the acceptable range of 4.5–6.5. Prior to the cycling test, Formula 1 had a pH of 4.98, Formula 2 had a pH of 5.16, Formula 3 had a pH of 5.39, and the negative control exhibited a pH of 5.01. After the cycling test, the pH values slightly increased to 5.01 for Formula 1, 5.27 for Formula 2, 5.48 for Formula 3, and 5.03 for C-. The slight increase in pH observed after the cycling test may be attributed to the decomposition of certain components during storage, as well as the influence of environmental factors and storage temperature. Nevertheless, the pH values remained within the acceptable range for topical cream formulations (4.5–6.5). Statistical analysis using a paired samples t-test showed no significant difference in pH values before and after the cycling test ($p = 0.066$, $p > 0.05$), indicating that the formulations maintained pH stability during the stability testing period [43].

The spreadability test was conducted to determine the ability of the yellowfin tuna (Thunnus albacares) skin extract cream to spread evenly when applied to the skin surface. After one minute of testing, the diameter of cream spread was measured. The acceptable spreadability range for topical cream formulations is 5–7 cm. Good spreadability enhances the contact area between the cream and the skin, thereby facilitating faster absorption of the active ingredients (43, 44). The spreadability results prior to the cycling test showed that Formula 1 had a spread diameter of 5.68 cm, Formula 2 of 5.47 cm, Formula 3 of 5.23 cm, and the negative control of 5.99 cm. After the cycling test, Formula 1 exhibited a spreadability of 5.71 cm, Formula 2 of 5.53 cm, Formula 3 of 5.26 cm, and the negative control of 6.00 cm. As shown in Table 6, all cream formulations and the cream base (C−) fell within the acceptable spreadability range of 5–7 cm. Statistical analysis using the paired samples t-test revealed no significant difference in spreadability before and after the cycling test ($p = 0.051$, $p > 0.05$). Although a slight increase in spreadability was observed following the cycling test, the changes remained within acceptable limits and did not deviate substantially from the required criteria [46].

Viscosity testing was performed to determine the consistency of the cream formulations using a viscometer, in which the spindle was immersed into the cream to a specified depth and rotated until a stable reading was obtained. Prior to the cycling test, the viscosity values were 5,299 mPa·s for Formula 1, 8,180 mPa·s for Formula 2, 12,839 mPa·s for Formula 3, and 4,500 mPa·s for the negative control. After the cycling

test, viscosity values slightly decreased to 5,200 mPa·s for Formula 1, 7,780 mPa·s for Formula 2, 12,380 mPa·s for Formula 3, and 4,380 mPa·s for the negative control. As presented in Table 7, all formulations exhibited a trend of decreasing viscosity following the cycling test. This reduction may be attributed to temperature effects, as increased temperature can weaken intermolecular forces by increasing the distance between molecules, thereby reducing formulation viscosity. Nevertheless, the viscosity values of all formulations remained within the acceptable range of 2,000–50,000 cPs (1 cPs = 1 mPa·s). Statistical analysis using the paired samples t-test indicated no significant difference in viscosity before and after the cycling test ($p = 0.063$, $p > 0.05$), demonstrating that the observed changes did not compromise formulation stability.

The cream type test was conducted to ensure that the formulations remained stable and did not undergo phase inversion during the cycling test. Water-in-oil (W/O) creams consist of water as the dispersed phase and oil as the continuous phase, stabilized by polyvalent ions with polar groups, resulting in a greasier texture and higher viscosity compared to oil-in-water (O/W) creams. In contrast, oil-in-water (O/W) creams consist of oil droplets dispersed within an aqueous continuous phase. Based on the results presented in Table 8, all formulations were identified as O/W (M/A) type both before and after the cycling test. This was confirmed by the uniform distribution of blue coloration following the addition of methylene blue reagent, indicating that water was the external phase of the formulation [27].

The anti-aging activity of a topical product is determined by the extent of wrinkle formation induced by UV exposure on the skin. An increased number or severity of wrinkles indicates insufficient anti-aging activity of the formulation [47]. In this study, the anti-aging activity of the yellowfin tuna (Thunnus albacares) skin extract cream was evaluated by exposing rats to UV-B radiation at a distance of approximately 10 cm for 17 minutes per day, five days per week for two weeks. Following UV-B irradiation, the cream formulations were applied to the dorsal skin of the rats, and wrinkle severity was observed over a period of 10 days using a scoring system: 0 = normal skin texture; 1 = presence of shallow wrinkles; and 2 = presence of deep wrinkles accompanied by skin laxity. Higher scores indicated lower anti-aging efficacy of the cream. The experimental design consisted of five treatment groups: negative control (cream base without active ingredient), collagen cream formulations at concentrations of 1%, 3%, and 5%, and a positive control using a commercial product (Wardah anti-aging cream) (47, 46).

After assessing wrinkle scores in replicate rats I, II, and III, the most frequently occurring (dominant) score for each day was determined using descriptive analysis in Microsoft Excel with the formula "=MODE.SNGL(range)" for each treatment group. The data were subsequently visualized in graphical form to illustrate the distribution and trends of wrinkle scores, as presented in Figure 1.

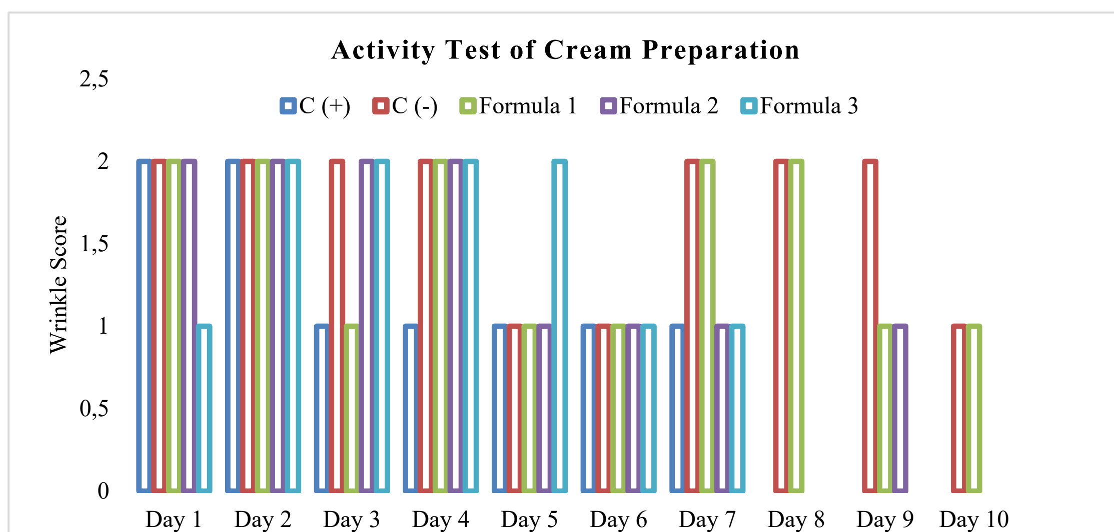

Figure 1. Anti-aging activity of yellowfin tuna (Thunnus albacares) skin extract cream in Wistar rats (Rattus norvegicus)

Wrinkle Scoring Criteria:
0 = normal skin texture
1 = presence of shallow wrinkles
2 = presence of several deep wrinkles accompanied by skin laxity

ISSN : 1907-8153 (Print)
e-ISSN : 2549-0567 (Online)

The observation results demonstrated that in the positive control group, wrinkle scores decreased from 2 (deep wrinkles) on days 1–2 to 1 (shallow wrinkles) on days 3–7, and reached 0 (normal skin texture) on days 8–10, indicating a progressive improvement in skin texture. In contrast, the negative control group showed persistence of a wrinkle score of 2 until day 4, followed by a decrease to score 1 on days 5–6. However, the wrinkle score increased again to 2 on days 7–9 and decreased to 1 on day 10. These findings indicate that the cream base without active ingredients was less effective in reducing wrinkle formation.

The anti-aging activity test results revealed that in Formula 1 (1%), wrinkle scores fluctuated between 2 (deep wrinkles) and 1 (shallow wrinkles) throughout the 10-day observation period, suggesting an unstable improvement in skin condition. In Formula 2 (3%), the initial wrinkle score of 2 persisted until day 4, decreased to 1 on days 5–7, and reached 0 (normal skin texture) on day 8. Although shallow wrinkles reappeared on day 9 (score 1), the skin condition returned to normal (score 0) on day 10, indicating better anti-aging activity compared to Formula 1. Formula 3 (5%) demonstrated the most prominent anti-aging effect. Following UV-B exposure, shallow wrinkles (score 1) were observed on day 1, which progressed to deep wrinkles (score 2) on days 2–5. However, gradual improvement was observed on days 6–7 (score 1), and normal skin texture (score 0) was achieved on days 8–10. Overall, Formula 3 was the most effective in improving skin texture and reducing wrinkle severity compared to Formulas 1 and 2 as well as the negative control, with results approaching those of the positive control. These findings indicate that higher collagen concentrations are associated with greater anti-aging activity, particularly in maintaining skin hydration and reducing visible signs of aging [47, 48].

The results of this study demonstrate that anti-aging cream containing yellowfin tuna (Thunnus albacares) skin extract effectively improves skin aging parameters, particularly by reducing the severity and depth of wrinkles in Wistar rats. The transition of wrinkle scores from deep wrinkles to shallow wrinkles and eventually to normal skin texture reflects a clinically relevant improvement in skin condition following continuous cream application. These findings are consistent with previous reports suggesting that marine collagen-based active ingredients play a crucial role in maintaining dermal structural integrity and slowing the skin aging process [26, 47].

The reduction in wrinkle depth is thought to be associated with improved dermal structural integrity, possibly related to collagen supplementation effects. Marine-derived collagen peptides have been reported to stimulate fibroblast activity in previous studies, and a similar mechanism may occur in the present model [37]. This mechanism supports the present findings, indicating that the application of yellowfin tuna skin extract cream contributes to the gradual restoration of skin structural integrity.

In addition, Based on previous studies on marine collagen, this effect may be associated with reduced oxidative stress and potential modulation of matrix metalloproteinase (MMP) activity, although these parameters were not directly measured in the present study [34]. The bioactive compounds present in yellowfin tuna skin extract are presumed to exhibit antioxidant properties that help suppress this process, thereby slowing the formation of new wrinkles while promoting the improvement of existing wrinkles.

The findings of this study are also consistent with previous research on marine collagen-based anti-aging cream formulations, which reported improvements in wrinkle-related parameters following topical application over a defined period. Earlier studies demonstrated that regular use of marine collagen creams effectively reduced wrinkle depth and enhanced skin surface smoothness, although the observed improvements were gradual and dependent on the duration of application [30]. This observation aligns with the results of the present study, in which wrinkle score improvement occurred progressively throughout the observation period, as illustrated in the corresponding figure.

Figure 2. Treatment of the dorsal skin of Wistar rats

Description:
A = Dorsal skin before UV-B exposure, no wrinkles observed
B = Dorsal skin after UV-B exposure, wrinkle formation observed
C = Dorsal skin after application of yellowfin tuna skin extract cream, wrinkles diminished

https://doi.org/ 10.32382/medkes.v21i1

The transition of wrinkle scores from deep wrinkles to normal skin texture indicates that the anti-aging effect is not instantaneous but requires sufficient duration of application. Miranda, emphasized that wrinkle improvement resulting from marine collagen is generally cumulative, as it is associated with dermal remodeling processes that require time to occur optimally [45]. Accordingly, the findings of this study reinforce the understanding that treatment duration is a critical factor in the success of topical anti-aging therapy[23].

Within the context of animal models, a clinically significant reduction in wrinkle formation serves as an important preliminary indicator prior to further evaluation using histopathological parameters or molecular biomarkers. Cruz, reported that macroscopic observation of skin wrinkles in animal models remains a relevant and widely used initial parameter for assessing the effectiveness of anti-aging agents, particularly in topical formulation studies [25]. Therefore, the results of the present study can be considered valid as preliminary evidence of the anti-aging activity of yellowfin tuna skin extract.

Overall, the findings of this study are consistent with research published over the past five years demonstrating that collagen and bioactive compounds derived from marine sources possess substantial potential in improving skin aging parameters, particularly in reducing wrinkle severity and depth. Consequently, anti-aging cream formulated with yellowfin tuna (Thunnus albacares) skin extract shows strong potential for further development as an effective and applicable marine-based natural anti-aging product within the field of cosmetic and pharmaceutical sciences.

LIMITATIONS

Several limitations of this study should be acknowledged. First, the relatively small sample size may limit the statistical power and generalizability of the findings. A larger experimental group would provide more robust evidence regarding the observed anti-aging effects.

Second, the primary endpoint was based on macroscopic wrinkle scoring, which, although practical, may be subject to observational variability. The inclusion of histological analysis or quantitative skin elasticity measurements would provide more objective confirmation of dermal structural changes.

Third, the study did not incorporate blinding procedures or biomarker assessments such as collagen density, matrix metalloproteinase (MMP) activity, or oxidative stress markers. The absence of these molecular parameters limits mechanistic interpretation of the observed improvements.

Finally, although UV-B exposure was administered to induce photoaging, the exact cumulative UV dose absorbed by the skin was not quantitatively measured. Variability in irradiation intensity or exposure uniformity may influence the degree of skin damage and treatment response. Future studies should include standardized dosimetry to improve experimental precision.

CONCLUSION AND RECOMMENDATIONS

The topical formulation containing yellowfin tuna skin collagen demonstrated a reduction in wrinkle scores in a UV-B-induced photoaging model, with the 5% concentration showing the most pronounced improvement. These findings suggest a potential dose-dependent effect of marine-derived collagen in improving skin appearance under photoaging conditions. However, the outcomes of this study were based primarily on macroscopic visual scoring without histological or molecular confirmation. Therefore, the biological mechanisms underlying the observed effects cannot be conclusively determined. Future research should incorporate larger sample sizes, blinded assessments, quantitative skin elasticity measurements, histological evaluation of dermal collagen density, and molecular biomarkers such as MMP expression and oxidative stress indicators. Additionally, standardized UV dosimetry and optimized extraction parameters should be considered to strengthen experimental validity and mechanistic interpretation.

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