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Original Article

PHYSICAL STABILITY AND SAFETY TESTING OF COSMETIC FORMULATIONS CONTAINING COLLAGEN FROM CATFISH (*PANGASIUS* SP.) SWIM BLADDER

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ABSTRACT

Objective: Collagen is a fibrous protein that confers strength and flexibility on bone and other body tissues, such as the skin and tendons, and is the main component of the extracellular matrix. It is used in cosmetics for its anti-aging activity. The primary source of commercial collagen is pig skin, but its use is imperfect due to halal concerns and the risk of biological contamination. Therefore, this research was conducted to identify alternate sources of collagen derived from fish. This study used the swim bladders of catfish that were not consumed and sought to separate the collagen from and use it in a safe anti-ageing serum for humans.

Methods: Three formulations of 1%, 2%, and 3% collagen were developed in the laboratory using experimental methods. The three formulations were then evaluated for physical stability and *in vivo* irritation.

Results: Physical quality testing showed that the 1%, 2%, and 3% formulations remained stable at room, low, and high temperatures. However, in the irritation test, the 2% and 3% formulations resulted in mild erythema after 24, 48, and 72 h under constant conditions.

Conclusion: The 1% formula was confirmed stable and safe.

Keywords: Catfish, Collagen, *In vivo*, Formulation

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INTRODUCTION

Collagen is a group of water-insoluble proteins that comprise 30% of all proteins in the human body. In the human body, collagen is an organic structure for building bones, teeth, joints, muscles, and skin. At least 1% of the collagen in the human body is lost every year; at the age of thirty, humans lose about 15–20% of their collagen, and at the age of 40, humans do not produce collagen anymore, so the collagen loss reaches 35–40% [1].

This decrease in the amount of collagen is also related to the oestrogen hormone, which plays a role in converting fibroblasts into collagen. Damage to collagen in the skin can be caused by exposure to UV-A and UV-B radiation in sunlight. Because the collagen content of the human body decreases with age [2], a solution is needed to reduce the negative effects of this change, such as the application of collagen. Collagen is an important protein that is produced in the human body and the main collagen structures are found in the skin, tendons, and bones. The term 'collagen' comes from the Greek 'kola', which means 'gum', and 'gene', which means 'production'. Collagen is recommended for functional biomaterials. It has low immunity power and high biocompatibility, which are good parameters for studying polymers for biomedical purposes, such as cosmetic pharmaceutical production [3]. Collagen is also safe and effective to use as a biomaterial in film technologies and clinical practice. It is used in dentures, skin regeneration, and biodegradable matrices and has been used in the fields of cardiac surgery, plastic surgery, orthopaedics, urology, neurology, and ophthalmology. Collagen also has many benefits in the food industry because it contains high protein and has useful properties, such as the ability to absorb water and form emulsions [4].

Vertebrates contain many collagens, which are useful as biomaterials in the pharmaceutical and medical industries. Currently, collagen is extracted from animals and cadavers, particularly human adipose tissue, which remains in the stomach after liposuction. We selected human adipose tissue, which is routinely left behind after liposuction, as an abundant source of human collagen [5]. Human collagen was obtained from adipose tissue through two main steps, extraction from the extracellular matrix (ECM) by mashing, centrifugation, alkaline and alcohol treatment, and isolation from ECM-added pepsin dissolved in acetic acid. Pure collagen from human adipose tissue derivatives was characterized with Fourier transform infrared spectroscopy, polyacrylamide gel electrophoresis, amino acid analysis, and circular dichroism spectroscopy [6].

Collagen derived from fish has the advantage of high yield and no risk of disease transmission from marine animals or invertebrate fish. Mammalian collagen has higher thermal stability than fish collagen because the amino acid content of fish collagen is lower than mammalian collagen. Thermal stability is related to an organism's body temperature and living environment, and the low thermal stability of marine collagen limits its applications [7]. Collagen derived from fish skin and bones has a smaller molecular structure than collagen from beef or pork, making it easier to absorb. Collagen also has a high water absorption capacity, making it a good component for texturing, thickening, and gelling. In addition, it has properties related to surface behaviour, such as emulsion, foam formation, stabilization, adhesion and cohesion, protective colloid function, and film-forming capacity. Collagen is a good surface-active agent and can penetrate the lipid-free interface [8].

Collagen derived from fish is similar to bovine and porcine collagen in terms of amino acid composition and biocompatibility. Glycine is an amino acid that comprises more than 30% of the amino acids in collagen. In addition, the proportion of proline hydroxyl amino acids ranges from 35–48%, like that of mammalian collagen. These amino acids are essential for the formation of collagen's specific helical structure and its stability. However, low proportions of glycine were observed in some marine sources of collagen, such as the collagen sponge *Chondrosia reniformis*, which contains 18.9% glycine and 40% hydroxyproline. The same species was recently found to contain 31.6% glycine and 47.3% hydroxyproline. This difference can be explained by the fact that in addition to collagen, marine tissue contains other proteins, such as glycoproteins, which are strongly associated with collagen and appear as impurities that affect the purity of extracted collagen. In addition, this could be caused by structural and chemical differences between sources and various extraction methods and biochemical analyses used by researchers. Therefore, to obtain consistent results, strictly regulated conditions must be applied

for sample preparation and preservation before extraction [9]. Liu *et al.* stated that collagen could be isolated from swim bladders in yields close to those achieved by extracting it from bones, skin, and fish scales. Collagen from swim bladders has been extracted from *Arius parkeri, Cynoscion acoupa, Cynoscion leiarchus,* carp (*Hypophthalmichthys nobilis*), and snapper (*Lates calcarifer*) [10].

The unique function and role of the swim bladder are thought to be related to the structure of the connective tissue, particularly the ECM. The structural and functional molecules of ECM have not been fully characterized, but many components, such as elastin, laminin, fibronectin, and collagen have been extracted and used for various applications. Collagen is the main structural component of white connective tissue, which represents almost 25–30% of the total protein in the vertebrate body [11]. Collagen can be extracted and isolated via several methods, but acid extraction dominates because it is simple. The protein contained in fish swim bladders is predominantly collagen, which contains 35% glycine, about 11% alanine and proline, and about 21% hydroxyproline [12]. Collagen can be used in the food, cosmetic, biomedical, and pharmaceutical industries. Collagen can be used directly or converted into gelatine before being applied industrially. Collagen extracted from fish body parts has advantages, such as overcoming restrictions on the use of raw materials from terrestrial animals, such as pigs, cows, or chickens, among certain ethnic and religious groups [1].

Of the 25 types of collagens (I to XXV) that have been identified so far, types I and V have been identified in fish waste. Skin, bone, and fish scale waste contains type I collagen, while type V collagen is found in the connective tissue in the skin and tendons [1] (as well as other types of collagens that have not been identified in the swim bladder). According to Bhaskar and Mahendrakar, fish offal contains high levels of protein and unsaturated fat. Research conducted by Syarifudin examined the characteristics of the swim bladder collagen of yellowfin tuna; meanwhile, catfish swim bladders had the highest protein content at 25.67% [13].

Table 1: Water, ash, protein, and fat content of *Thunus albacares, Muraenesox talabon,* and *Pangasius* sp

Parameter	Percentage (%)		
	***Thunnus albacares* [1]**	***Muraenesox talabon* [2]**	***Pangasius* sp. [3]**
Water content	83.33	73.88	74.03
Ash content	0.29	0.27	0.08
Protein	12.09	24.74	25.67
Fat	1.44	0.50	0.03

MATERIALS AND METHODS

The swim bladders of catfish (*Pangasius* sp.) were collected from the fish fillet company PT Prosperous Partners Gift (Purwakarta West Java). NaOH (Merck, Germany), acetic acid (Merck, Germany), Oswald Durant viscometer (SI Analytics™ 285404014, Fischer, Sweden), analytical balance (Metrohm AG, Swiss), and shaker (Benchmark Scientific Orbi-Shaker, UK) were also gathered for experimentation and analysis.

Material preparation

The catfish swim bladders were sent directly from Purwakarta via a freight forwarder from PT Kurnia Mitra Makmur by land. The swim bladders that were sent were byproducts from fish filet processing the day before. The frozen swim bladders were packed in an ice box and covered with aluminium foil to keep them frozen until they arrived at the Pharmacy and Food Analysis Study Program in Jakarta. After arrival at the Ankosalkes Anafarma laboratory, they were immediately wet-sorted for further testing. The method used in this study involved stages of processing: 1) Raw material pre-treatment via wet sorting by sorting the swim bladders to be extracted into collagen. The selected swim bladders were intact and undamaged over the entire surface and were thick and in good condition. 2) Organoleptic wet raw material; after wet sorting, the swim bladders were cleaned by washing with tap water to remove any adhered material, including blood. 3) Isolation of wet raw material into collagen extract. The clean swim bladders were soaked with 0.1 N NaOH for 24 h, at a ratio of 1:10 wet swim bladders to NaOH. Soaking with NaOH removed proteins that dissolved in the solution other than collagen. 4) Extraction of swim bladders with glacial acetic acid for 24 h to hydrolyse the collagen from them. 5) pH testing of the collagen extract in acid. After the collagen extraction in acid was complete, the swim bladder collagen was cleaned by washing with tap water. Then, the pH of the washing water was tested until it registered as neutral using universal pH paper. 6) Organoleptic collagen extract, which consists of testing parameters for smell and appearance. 7) pH testing of the collagen extract using a pH meter (T.19000, Walk lab, UK). 8) Performing an assay of alkaline soluble protein with BSA–UV-Vis spectrophotometry (Shimadzu double beam). 9) Base formulation and dosage formulation with active substance content of 1%, 2%, 3%. 10) Testing the organoleptic preparations. 11) Preparation stability testing. 12) Safety testing of the preparation with *in vivo* irritation tests on three rabbits (2 females, 1 male) [2, 15].

Preparation of microemulsion serum formula

The microemulsion serum formula contained patented fish swim bladder extract (*Pangasius* sp.) as an active substance, propylene glycol, triethanolamine, and phenoxyethanol as surfactants (emulsifying agents), castor oils as the oil phase of the emulsion and as a preservative, and carbopol as a gelling agent, as well as fragrance and preservative [5].

Table 2: Pharmaceutical formulations

Materials	Formula I (%)	Formula II (%)	Formula III (%)
Water	90	89	88
Collagen swim bladder	1	2	3
Carbopol 960	0.5	0.5	0.5
Propylene glycol	5	4	3
Triethanolamine	1	1	1
Phenoxyethanol	1	1	1
Fragrance	0.5	0.5	0.5
HCO 40 hydrogenated castrol oils	1	1	1
Total	100	100	100

Physical stability test of preparations

The organoleptic test was conducted by observing the new shape, colour, and appearance of the preparation. This test is important for observing preparations in general.

The pH test used universal pH paper. The preparation was dripped on universal pH paper and left for a few moments until a colour change occurred on the universal pH paper. Then, the results were compared to the scale on the universal pH paper, and the number observed was recorded as the pH of the preparation.

The viscosity test used the Oswald viscosity tool to measure kinematic and dynamic viscosity and the results were compared with the Indonesian National Standard viscosity data [2, 15].

Preparation safety test (irritation test) [2, 15]

The irritation test was conducted with the acute dermal method. The animal 5398/EDL/2023 used in this test was an adult, healthy male albino rabbit (*Oryctolagus cuniculus*) of the New Zealand White strain. Rabbits were chosen because their constituent cells and skin structure are similar to human skin cells. Male rabbits are preferred as models because they have more stable biological conditions than female rabbits, whose biological condition is significantly influenced by cycle time, gestation period, and lactation period. This is suggested by the OECD Guidelines 404 (accurate dermal irritation/corrosion).

The test animals used weighed 1–1.65 kg and were aged 2–3 mo. The rabbit food used was 'Vital' brand pellets with the following composition: moisture maximum of 10%, minimum protein 20%, maximum fat 5%, calcium 1.25%, phosphorus 1%.

Three male albino rabbits (*Oryctolagus curniculus*) from PPOMN BPOM with body weights of 1.2 kg were prepared for testing. They were acclimatized in the experimental room for 5 d and then placed in individual cages. Room temperature was around 20±3 °C and humidity was not more than 70%. The room lighting cycle was 12 h dark/12 h light. At least 24 h before testing, the rabbits' fur was sheared on their backs in an area of approximately 2.5 x 2.5 cm. The rabbits' fur was first cut with scissors, then shaved with a Gillette Vector razor to achieve smooth, hair-free skin. Shaving was conducted to avoid injuring the test animals. Each shaved area was surrounded by unshaven areas with clear boundaries. After shaving was complete, the test solution was administered. The serum dose was 0.5 g.

The acute dermal irritation test is conducted to determine whether the serum has an irritating effect on the skin and to assess and evaluate the characterization of a substance when applied to the skin. The acute skin irritation test was evaluated *in vivo* on test animals after epilation. The sample to be tested was smeared on the skin of the test animal. The appearance of oedema or erythema due to a reaction to the sample was evaluated at 1, 24, 48, and 72 h after application. A scoring system was used to classify the type of test substance from non-irritant to very irritant. In the acute dermal irritation test, six groups were created: negative control in the form of serum that did not contain catfish swim bladder collagen extract, positive control in the form of serum circulating in the community, and normal control as a comparison in the form of untreated areas, as well as three test groups for three formulas with each formula containing catfish swim bladder collagen extract with concentrations of 1%, 2%, and 3% each in an area of±6 cm^2.

If the test preparation was suspected to be irritating, the test was conducted using one test animal with three exposure patches each for the three formulas and three patches each for the three control conditions. The first patch would be lifted after 3 min; if no serious skin irritation occurred, then the second patch would be lifted after 1 h; if there was no severe skin irritation, then the third patch would be lifted after 4 h and the gradation of skin injury would be determined. If irritation was seen after 3 min or 1 h, the test would be stopped and all patches removed.

Observations would continue for 14 d unless irritation occurred at the start of the test. If no irritation was seen after 4 h of exposure, then the test would be continued by adding two additional animals, each of which would be exposed for 4 h [2, 15].

If the test preparation was suspected to be non-irritating, the test would be conducted using a test animal and one patch for each condition with an exposure period of 4 h. After 4 h; the exposure residue would be removed with water or another solvent. These test animals would be observed for the presence or absence of erythema and oedema. The response assessment would be conducted at 1, 24, 48, and 72 h after lifting the patch. If skin damage could not be identified in the form of irritation or damage at 72 h, observations could be continued until Day 14 to determine reversibility [2, 15].

The test animals underwent a series of tests: each test animal received the same treatment with the same variable – the concentration of the active substance. Three test animals were used, each receiving six treatments as detailed in fig. 1.

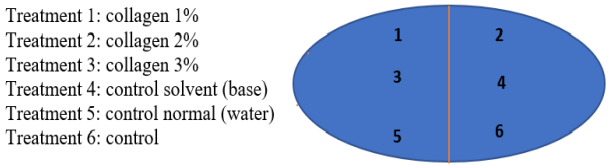


Fig. 1: Rabbit shaving

RESULTS AND DISCUSSION

Pre-treatment wet sorting

The raw material pre-treatment wet sorting process was shown in fig. 2.

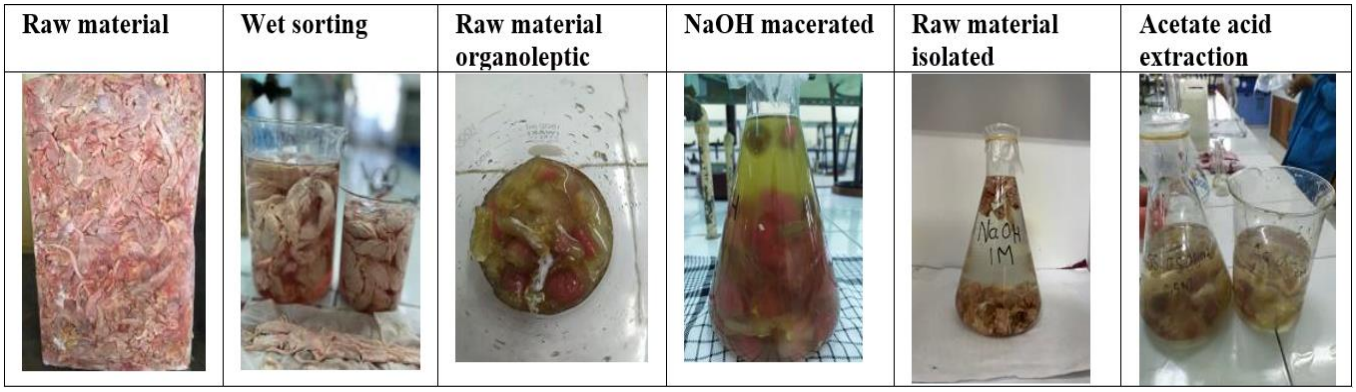


Fig. 2: Raw material pre-treatment process

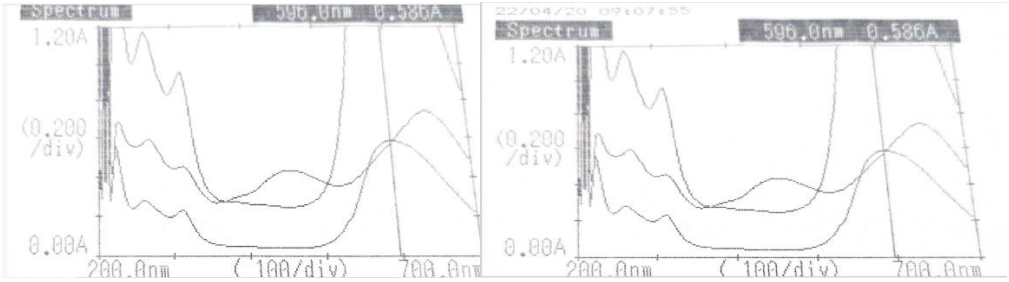


Fig. 3: Alkaline soluble protein spectrum

Table 3: Serum-based formulations

Material	Percentage
Water	
Fish swim bladder	
Carbopol 960	1
Propylene glycol	5
Triethanolamine	1
Phenoxyethanol	1
Fragrance	1
HCO 40 hydrogenated castrol oils	1
Total	100%

Base formulation

The base consists of ingredients that are formulated into a serum base that has physical stability and a pH that meets the standards for a serum base.

Physical stability test of preparations

A cycling test was conducted to test the stability of the serum during storage at extreme temperatures (Rieger, 2000). The test was performed by storing the serum at 4±2 °C for 24 h and then transferred to the oven at 40±2 °C for 24 h. This treatment was called one cycle and the cycle was conducted six times to clarify the changes in the serum. After the serum cooled, water was released in the serum, but if the well-formed film remains functional under the conditions induced by ice before coalescence, then the system will remain stable. Then, the organoleptic test, pH test, viscosity test, and homogeneity test were carried out.

Table 4: Organoleptic test of preparations

No.	Formula	Day 0	Day 12
1	Base	Form: Semi-solid Colour: Clear Smell: Characteristic aroma	Form: Semi-solid Colour: Clear Smell: Characteristic aroma
2	preparation 1%	Form: Semi-solid Colour: Clear Smell: Characteristic aroma	Form: Semi-solid Colour: Clear Smell: Characteristic aroma
3	preparation 2%	Form: Semi-solid Colour: Clear Smell: Characteristic aroma	Form: Semi-solid Colour: Clear Smell: Characteristic aroma
4	preparation 3%	Form: Semi-solid Colour: Clear Smell: Characteristic aroma	Form: Semi-solid Colour: Clear Smell: Characteristic aroma

Table 5: pH test at low term to high term day 0 to day 12

No.	Formula	Day 0	Day 12
		Low term High term	Low term High term
1.	Base	4.5±0.024.5±0.02	4.5±0.02 4.5±0.02
2.	Preparation 1%	4.5±0.024.5±0.02	4.5±0.02 4.5±0.02
3.	Preparation 2%	4.5±0.024.5±0.02	4.5 ±0.02 4.5±0.02
4.	Preparation 3%	4.5±0.024.5±0.02	4.5±0.02 4.5±0.02

The data are presented as mean±SD, n=3

pH testing was conducted to determine whether the preparation had the same pH as the skin; pH testing is also useful to determine the safety of the preparation in use. The pH of the serum must match the pH of the facial skin, i. e. 4.5 to 6, because unmatched pH levels can result in skin irritation. The results of the pH test of the 1%, 2%, and 3% preparations at low temperatures were 4.5 from day 0 to day 12, within the pH range of the skin.

The viscosity test measures the resistance of a liquid or fluid to flow; the thicker a preparation is, the greater the amount of energy required for it to flow. According to SNI 16-4399-1966, the standard viscosity of serum is 800–1000cps. This standard viscosity value is greatly influenced by several elements, such as the excipients in the formula (polymers, surfactants, thickening agents), the choice of surfactant, and the proportion of dispersed and dispersing phases [3, 14]. If a preparation is overly viscous, it will be difficult for the active substance to separate from the base, as well as difficult to apply to the skin. If the preparation is too dilute, it will shorten the contact time of the preparation with the skin, resulting in suboptimal absorption of the active substance.

Homogeneity test

Homogeneity is an important factor and a measure of the quality of the preparation because it indicates that the active substances used have

been mixed evenly. The results of the tests that were conducted on serum preparations for the three replications are presented in table 7.

Table 6: Viscosity test at low and high temperature

No.	Formula	Day 0	Day 12
		Low term High term	Low term High term
1.	Base		
2.	preparation 1%	17140 cp 32228 cp 34311 cs 40744 cs	27470 cp 26489 cp 34728 cs 33488 cs
3.	preparation 2%	22492 cp 25606 cp 28435 cs 32372 cs	19229 cp 19425 cp 24309 cs 24557 cs
4.	preparation 3%	20633 cp 20529 cp 26085 cs 25953 cs	18836 cp 1.8983 cp 23183cs 23999 cs

Note: Cp: dynamic viscosity, cs: kinematic viscosity

Table 7: Homogeneity tests

	Formula			
	Base	Preparation 1%	Preparation 2%	Preparation 3%
Day 0	 Homogeneous	 Homogeneous	 Homogeneous	 Homogeneous
Day 12	 Homogeneous	 Homogeneous	 Homogeneous	 Homogeneous

This homogeneity test was conducted to determine whether the active substances and ingredients used are mixed well; that is, the preparation must show a homogeneous composition and the absence of coarse grains [2]. Homogeneity can be observed by placing swim bladder collagen extract serum preparations between two object glasses and looking for the presence of coarse particles or inhomogeneity under light. This is because the requirement for homogeneity is that the preparation should not contain coarse material that can be felt [2]. This observation was performed on serum preparations that included swim bladder collagen extract at concentrations of 1% (Formula I), 2% (Formula II), and 3% (Formula III), which all showed a homogeneous distribution and no coarse particles.

Table 8: Spreadability test

No.	Formula	Day 0	Day 12
1	Base	3.33±0.40	3.33±0.40
2	Preparation 1%	3.23±0.25	3.43±0.31
3	Preparation 2%	3.43±0.45	3.43±0.60
4	Preparation 3%	3.63±0.31	3.80±0.30
5	Normal	5.40±0.66	5.40±0.66
6	Control	5.23±0.55	5.23±0.55

The data are presented as mean±SD, n=3

The spreadability test measures the ease of spreading the preparation on the skin at a certain time. This parameter is important to measure because topical products must be easily applied between 0 and 12 d after storage. The spreadability of the basic formula, serum 1%, 2%, 3%, and the normal and comparison controls on Day 0 to Day 12 was obtained and ranged from 3.33-3.33 cm; 3.23-3.43 cm; 3.43-3.43 cm, 3.63-3.80 cm, 5.40-5.40 cm, 5.23-5.23 cm, respectively. The 3% serum formula exhibited better spreadability than the 2% and 1% serum formulas. On the 12th day, there was a slight increase in the spreadability of the three formulas. Spread power: the addition of pressure increases the diameter of the spread area.

Skin irritation test

To guarantee the safety of the product in use, topical preparations must be tested for irritation using test animals. Rabbits are used for irritation tests because rabbit skin is more sensitive than human skin. In the irritation test, a dose of 1%, 2%, and 3% samples was applied to a 1 x 1-inch skin (table 9). The data from the acute dermal irritation test results were analysed using a skin irritation score for erythema and oedema that describes the severity of the wound on each animal at 24, 48, and 72 h after the patch was lifted. The irritation score (primary irritation index) of the test preparation is a combination of all observations from the test. The primary irritation

index can be calculated using the primary irritation index formula [2, 15].

A = Sum of erythema and oedema scores for all sample observation points at 24, 48, and 72 h divided by the number of observations

B = Sum of erythema and oedema scores for all control observation points at 24, 48, and 72 h

C = Number of animals

Acute dermal irritation test

The acute dermal irritation test is one of a series of toxicity tests conducted on test animals (albino rabbits) to detect toxic effects that appear after exposure to test preparations [15]. The data obtained can be informative about the degree of danger if the test preparation is applied to humans so that the safe dosage can be determined. The purpose of the acute dermal irritation test is to detect irritating effects on the skin and to assess and evaluate the characteristics of a substance when applied to the skin [15].

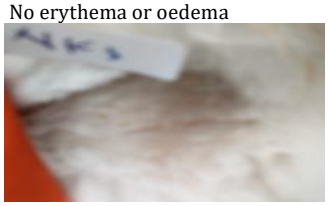
Three albino rabbits, *Oryctolagus cuniculus*, of a New Zealand strain were used, two females and one healthy, mature male. Both male and female rabbits were used because the incidence of acute irritation to the skin can occur in both sexes [16]. The rabbits' weights were 1 to 1.65 kg to reduce the variability of the effect due to differences in body weight. The first rabbit weighed 1.45 kg; the second, 1.65 kg; and the third, 1.50 kg. The rabbits were acclimatized for 5 d in the rabbit-rearing laboratory before they were used according to the provisions of BPOM RI Regulation Number 7 of 2014 concerning the guidelines for non-clinical toxicity tests [2, 15].

One day before treatment, the rabbits' fur was shaved over an area of 10x15 cm with scissors and manual hair scraping to ensure that the catfish swim bladder collagen serum preparation would be applied to the rabbits' skin and be absorbed properly. A dose of 0.5 g of catfish swim bladder collagen serum was applied to the rabbits' skin over an area of approximately 6 cm^2 (2x3 cm). The application was performed 24 h after epilation, and the area was covered with gauze and glued with a non-irritant plaster. Then, the area was observed for erythema or oedema.

Erythema is a reddish reaction on the skin that arises as a side effect of using topical preparations. This redness is also marked by the appearance of prominent, symmetrically arranged spots. In addition to erythema (redness), irritation symptoms include vesiculation (wateriness) and itching and burning [2, 16]. Oedema is a swelling reaction on the skin that arises as a result of the side effects of using topical preparations. Oedema occurs due to the increased volume of fluid outside the cells and blood vessels, which accumulates in the body's tissues [2, 16].

Qualitative and quantitative observations were made. Qualitative observation was performed by observing whether or not erythema and oedema effects arose after test preparation application to the rabbits' skin. Quantitative observations were conducted by grouping the effects of erythema and oedema that arise according to the scores in table 8.

Table 9: Irritation test

Formula	24 h	48 h	72 h
	Erythema with oedema	**Erythema with oedema**	**Erythema with oedema**
Control			
	No erythema or oedema	No erythema or oedema	No erythema or oedema
Preparation 1%	No erythema or oedema	No erythema or oedema	No erythema or oedema
			
Preparation 2%	Erythema but no oedema	Erythema but no oedema	Erythema but no oedema
			
Preparation 3%	Erythema but no oedema	Erythema but no oedema	Erythema but no oedema
			

CONCLUSION

Based on the findings, the following conclusions can be drawn: The physical quality testing in this study of formulas I, II, and III indicated that they are stable at room temperature low, and high temperatures; however, in the irritation test, the 2% and 3% formulas resulted in very minor erythema at 24, 48, and 72 h under constant conditions. Therefore, only formula I is considered a stable, safe formula.

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AUTHORS CONTRIBUTIONS

Misde Yola contributed to the design and implementation of the study, the analysis of the results, and the writing of the manuscript. Susy Saadah conducted the experiments, formulated serum preparations, tested the physical quality of the preparations, and wrote the manuscript. Deni Rahmat supervised the project. All authors discussed the results and commented on the manuscript.

CONFLICT OF INTERESTS

The authors declare no conflicts of interest.

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