Comparison of blasting cream anadara granosa with mytilus viridis in white rat skin (rattus novergicus)

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Abstract. Collagen is the result of determination from chitosan blood shells (Anadara granosa) and green shells (Mytilus viridis). Previous research, extraction was carried out to obtain collagen. The yield of collagen produced is 2.03% with a concentration of acetic acid 0.75 M. Collagen can penetrate the main components of the dermis skin layer (the bottom of the epidermis) made by fibroblast cells. Based on the results of previous studies, the collagen produced will be made into a dosage form so that it can be better utilized. Based on the description above, researchers are interested in comparing blood clam shells and green mussel shells with a study entitled the comparison of physical test blasting creams of Anadara granosa with green shells Mytilus viridis against the skin of male white rats. Laboratory experimental research using a post test control design research design in the form of post only design which is divided into 5 treatment groups with each group consisting of 5 male white rats. The negative control group by giving a base cream and the test group by giving blasting cream Anadara granosa with Mytilus viridis concentrations of 10%, 15%, 20% and 25%. The parameter observed was skin moisture level. The results of statistical analysis using the ANOVA test. The results of this study are blasting cream Anadara granosa and Mytilus viridis can provide a moisturizing effect on rat skin. The most optimal concentration as a moisturizing effect on rat skin is a concentration of 25%.

1. Introduction

Utilization of marine products in Kendal has been maximized, but the utilization of waste has not been maximized for daily income needs. Coastal communities only consume clam meat and do not utilize waste from shellfish. Shells from shells mostly only become waste and there is no utilization or as a form of craft [1]. In fact, meat and shellfish can be processed into beauty products and sources of vitamins for the body. Blood clam meat contains nutrients and minerals such as vitamin B1, iron, zinc, selenium as well as a source of collagen. The content in the clam shell in the form of Ca + C 98.7%, Mg 0.0476%, Na 0.9192%, P 0.0183%, K 0.0398% and more than 71% of collagen. Collagen can be used in industrial fields such as facial, hair, body care products such as shampoo, conditioner, soap and other cosmetics. Collagen as a body treatment, especially on the skin is useful as a thickener and makes the skin look firmer, the process to get collagen must be through extraction [2]. Previous research, extraction from kitin was carried out to obtain collagen. The yield of collagen produced is 2.03% with a concentration of acetic acid 0.75 M. The immersion of collagen is influenced by the concentration of acetic acid. The higher the concentration of acetic acid, the greater the yield of collagen [3]. Collagen can penetrate the main components of the dermis skin layer (the bottom of the epidermis) made by fibroblast cells. Based on the results of previous studies, the collagen produced will be made into a dosage form so that it can be better utilized.
Based on the description above, researchers are interested in comparing blood clam shells and green mussel shells with a study entitled the comparison of physical test blasting cream of blood clam shells (Anadara granosa) with green shells (Mytilus viridis) against the skin of male white rats (Rattus novergicus).

2. Experimental
2.1 Materials
Removal of non-collagen protein. The clam shell is cleaned. The sample was then weighed in a ratio of 1:20 (w / v) and then immersed in 0.1 M NaOH solution for 24 hours. Samples are filtered using filter paper, then washed with distilled water until the sample pH approaches or reaches pH 7.

2.2 Method
Collagen extraction was done by immersion in acetic acid according to modified Muyonga et al. (2004). The sample was weighed twice, with a ratio of sample weight and volume of solution 1:10 (w / v), then given the treatment code A. Treatment A was macerated in 0.75 M acetic acid macerated for 3 days. The result of extraction of treatment A was filtered and then salted out by adding NaCl 0.9 M. Then it was centrifuged for 10 minutes at a speed of 10,000 rpm to precipitate the wet collagen residue fibers. The separation results are then filtered and roasted at 60°C for one day to get dry collagen. The dried collagen is then weighed to calculate the amount of yield obtained.

2.3 Yield measure collagen
The dried collagen obtained is prepared, then weighed and the results recorded. The yield calculation can be seen in equation (1).

\[
\text{Yield \%} = \frac{\text{dry collagen weight}}{\text{powder weight}} \times 100\%
\]  

(1)

2.4 Making cream preparations
The formulation of blood clam shell collagen cream preparations made four preparations, each 30 g with a variety of the amount of collagen (active substance). Literature from the Indonesian Formulary [4] with the following formulation in table 1 and 2.

| Table 1. 150 gram Cream Base Material Formulation |
|-----------------------------------------------|
| Material            | Formula | Amount   | Efficacy         |
|---------------------|---------|----------|-----------------|
| Asam stearat        | 15%     | \(\frac{15}{100}\) \(\times 150 = 22,5\) | Emulsifying     |
| Vaselin album       | 25%     | \(\frac{25}{100}\) \(\times 150 = 37,5\) | Base            |
| Ćera alba           | 15%     | \(\frac{15}{100}\) \(\times 150 = 22,5\) | Emulsifying stabilizer |
| Trietanolamin       | 3%      | \(\frac{3}{100}\) \(\times 150 = 4,5\) | Emulsifying agent |
| Propilenglikol      | 15%     | \(\frac{15}{100}\) \(\times 150 = 22,5\) | Humectant       |
| Nipagin            | 0.2%    | \(\frac{0.2}{100}\) \(\times 150 = 0,3\) | Preservative    |
| Aquadest            | ad 150  | 150-(22,5+37,5+22,5+4,5+22,5+0,3) = 40,2mL | Solvent         |
2.5 Evaluate cream preparations

Cream evaluation is carried out at the beginning of manufacture and after one month of storage, cream preparations observed include:

1) Organoleptic

The preparations that have been made are made observations on the appearance of the preparations including odor, color and consistency of the preparations. Organoleptic testing of color and consistency was repeated for each formula five times each.

2) Homogeneity

Homogeneity test is done by means of a cream weighed ± 0.1 g then placed on a glass object and covered with other glass objects, then the cream preparation is given a load of 1 kg for 5 minutes, after which it is observed with a magnifying glass. Homogeneity testing was repeated for each formulation five times each.

3) Scattering power

The scatter power test is done by placing ± 0.5 g of cream on a scale glass plate and on top of it covered with the same glass, then given a weight of 50 g and 100 g for 2 minutes, then measured the size of the spread diameter formed (cm). The spread test was repeated on each formula five times each.

4) Cream type testing

Cream type examination is done by giving one drop of methylene blue solution to 0.1 g of cream, then the spread of methylene blue in preparations under a microscope is observed. If the color is spread evenly on the cream preparation, it means that the type of cream is oil in water (M / A), but if the color is only in spots it means the type of cream is water in oil (A / M).

5) Testing the pH of the cream

The examination is carried out using a pH meter. The pH check is done by dipping the electrodes into 1 g of the cream preparation diluted with distilled water to 10 mL.

2.6 Preparation of mouse skin

The skin of male white rats used are male rats aged approximately 2 months, weighing 200-400 g. Rat are shaved on the back using a shaver. Rat that have been shaved, measured diameter of 2.8 cm. Shaved skin is oriented first. Prepare mice that have been shaved then smeared cream base as a negative control orientation. Collagen cream is applied to the skin of rats that have been shaved as much as 50 mg in each sample until the skin is covered with a layer of collagen cream. After that it is observed between the skin with a cream base and the skin that has been smeared with collagen cream, in the time and minute how many changes in shape, color, odor and skin moisture note the results.

2.7 Skin evaluation

a) Organoleptic examination

Organoleptic examination involves observing the shape, color, odor of the skin.

b) Skin moisture

Moisture measurement that is using a skin moisture meter (skin meter) before being given a cream and after being given a blasting cream.
3. Result and Discussion

3.1 Collagen Yield Levels

The yield from the extraction of *Anadara granosa* and *Mytilus viridis* samples using a concentration of 0.75 M can be seen in Table 3.

| Sample         | Initial weight | Dry collagen | Yield  |
|----------------|---------------|--------------|--------|
| *Mytilus viridis* | 900,0005 g    | 11,3023 g    | 1,227% |
| *Anadara granosa* | 900,0007 g    | 11,2003 g    | 1,257% |

From Table 3, shows the yield of collagen of *Mytilus viridis* 1.228% while *Anadara granosa* 1.257% means the concentration of acetic acid is best used 0.75 M based on previous research [3]. The concentration of acetic acid solution that can cause a decrease in protein levels because acetic acid will hydrolyze the peptide bonds more strongly so that there will be a loss of protein [5].

3.2 *Anadara granosa* and *Mytilus viridis* Collagen Cream Formulation

Collagen extract that has been produced from the extraction process is then used as ingredients for making cream. The resulting cream preparations are very good, non-sticky and homogeneous. Cream preparations in the form of thick solid white rather yellowish and easily applied to the intended object.

3.3 Test Results for *Anadara granosa* and *Mytilus viridis* Collagen Cream Evaluation

Cream evaluation test is an important element in determining the quality of preparations. Cream evaluation can be measured in blood clam shell collagen cream preparations including organoleptic, homogeneity, spreadability, pH test and type of cream. The results of characteristic testing can be seen in Table 4.

| Formula | Color         | Smell  | Dosage form | Homogenity      | Spread power | pH     | Cream type |
|---------|---------------|--------|-------------|------------------|--------------|--------|------------|
| **Base** | White         | Odorless| Solid       | No coarse grains | 3.45 cm      | 6.50   | M/A        |
| **F1**  | White         | Typical| Solid       | No coarse grains | 3.44 cm      | 6.53   | M/A        |
| **F2**  | Bone white    | Typical| Solid       | No coarse grains | 3.53 cm      | 6.50   | M/A        |
| **F3**  | Cream         | Typical| Oily solid  | No coarse grains | 3.64 cm      | 6.47   | M/A        |
| **F4**  | Yellowish     | Typical| Oily solid  | No coarse grains | 3.95 cm      | 6.45   | M/A        |

| Formula | Color         | Smell      | Dosage form | Homogenity      | Spread power | pH     | Cream type |
|---------|---------------|------------|-------------|------------------|--------------|--------|------------|
| **F1**  | White         | Typical    | Rather thick| Homogen          | 3.43 cm      | 6.50   | M/A        |
| **F2**  | Bone white    | Typical    | Rather thick| Homogen          | 3.55 cm      | 6.50   | M/A        |
| **F3**  | Cream         | Typical    | Rather soft | Homogen          | 3.66 cm      | 6.40   | M/A        |
| **F4**  | Yellowish     | Typical    | Rather soft | Homogen          | 3.98 cm      | 6.50   | M/A        |
| **Base** | White        | Typical    | Thick       | Homogen          | 3.53 cm      | 6.50   | M/A        |

Information:
Basis = Basis Krim
F1 = Collagen concentration 10%
F2 = Collagen concentration 15%
F3 = Collagen concentration 20%
F4 = Collagen concentration 25%

Organoleptic testing of blasting cream evaluation results was carried out with visual observations including color, odor and dosage form. The results of organoleptic testing of collagen shells in blood and green shells in table 4. show that formula 1 is white, formula 2 is bone white, formula 3 is yellowish white, formula 4 is yellowish and formula 5 is white based. The Mytilus viridis and Anadara granosa cream formulation of form 1, 2 and 5 is in the solid form while formulas 3 and 4 are in the oily solid form while in the green tap shells formula 1 and 2 are rather thick, formulas 3 and 4 are somewhat mushy. There is a difference in the dosage form in the cream because the more active substances (collagen) that are used the more the oil content contained in the cream will be more moist. Collagen concentrations of 10% and 25% more moisturize on a cream base than previous studies with a collagen concentration of 5% [6].

The results of scatter power testing can be seen in table 4.2. The spread test is the ability to spread the cream to the skin which is used to determine when the cream is applied. A good cream should be spread evenly when used on the skin. The spreadability of the creams from the 5 formulations showed good results, namely 3.45 to 3.97 for preparations of Anadara granosa while 3.45 to 3.55 for preparations of green shells. The difference in spreadability occurs because if there is the addition of other substances in an oily cream, the spread will be longer or wider in diameter produced.

Homogeneity test is very necessary in making a cream. The advantage of homogeneity tests is to find out which creams are made homogeneous and when used can be evenly distributed on the surface of the skin without any coarse grains. The test results from 5 formulations of the 2 samples showed good results ie there were no coarse grains.

Determination of pH is tested to determine the pH of the preparation made. The results of testing the pH of the 5 formulations of the 2 samples indicate that the pH obtained ranged between 6.41-6.56. From the results of the cream pH test in accordance with the cream pH range ranged from 4.5 to 6.5 [7].

3.4 Moisture Cream Collagen Test Results Against Mouse Skin

The results of the rat skin test were measured in humidity before being smeared with blasting cream using a moisture meter. The humidity test results can be seen in Table 5.

| Table 5. Mouse skin moisture test results |
|-----------------------------------------|
| Mean ± SD | Anadara granosa |
| Base ± SD | F1 ± SD | F2 ± SD | F3 ± SD | F4 ± SD | F5 ± SD |
| Day 1 | 10.3% 0.917 | 10.82% 0.208 | 11.46% 0.651 | 13.33% 0.361 | 15.94% 0.907 |
| Day 2 | 10.3% 0.652 | 13.02% 0.436 | 3.41% 0.650 | 15.53% 0.529 | 17.66% 0.755 |
| Day 3 | 10.3% 0.253 | 14.14% 0.513 | 14.46% 0.359 | 17.21% 0.529 | 19.44% 0.700 |
| Day 4 | 0.566 14.4% | 0.500 15.46% | 0.453 18.16% | 0.503 20.16% | 0.404 |
| Day 5 | 10.04% | | | | |
| Day 6 | 10.03% 0.301 | 13.18% 0.100 | 14.43% 0.356 | 16.86% 0.153 | 18.9% 0.208 |
| Day 7 | 10.2% 0.201 | 13.14% 0.265 | 13.43% 0.153 | 15.96% 0.503 | 18.3% 0.252 |
| Day 8 | 10.2% 0.415 | 12.12% 1.082 | 12.46% 0.250 | 14.86% 0.153 | 17.44% 0.416 |
Moisture cream is a way to ensure that preparations made can provide a moisturizing effect that is safe to use. Blasting cream test in this study was carried out using the topical method directly on white male rat test animals. The use of male rats was chosen so that the study of active substances on the surface of the rat's skin did not affect the mouse's hormones. The number of male rats used for the collagen cream test was 25 3-month-old rats with a body weight of 160-200 grams. Selection of this mouse. The number of rats used for the collagen cream test as a moisturizer was between 10-30 male rats [8]. Male rats were divided into 5 groups, namely the negative control group with cream base and 4 test groups with collagen cream from concentrations of 10%, 15%, 20%, and 25%. This research aims to learn more about the collagen used to moisturize mouse skin. The initial treatment of the rat's back is shaved first so that the hairs of the rat are not opened when applying blasting cream. The backs of the mice were shaved with a diameter of about 2.8 cm in each of the mice that would be used for trials. Furthermore, each rat measured its moisture before being smeared with collagen cream using a moisture test tool (skin moisture meter).

Moisture results from each rat's skin before smeared with collagen cream were replicated during 5 tests, but the data used 3 tests were seen from the best results. The average results per day of initial skin moisture of rat on the basis of the cream obtained results are day 1 (10.5%), day 2 (10.3%), day 3 (10.1%), day 4 (10.03%), 5th day (10.01%), 6th day (10.0%), and 7th day (10.0%) for a sample of a blood clam while the results of a sample of a green mussel shell are a day 1st (10.6%), 2nd day (10.2%), 3rd day (10.1%), 4th day (10.04%), 5th day (10.01%), day 6th (10.0%), and the 7th day (10.0%). The process of reducing moisture on the skin of mice is due to the cream base reacting at the beginning of the application only because the treatment in this study was done only with one time applying the base to the skin and the treatment was seen from day 1 to day 7. When the initial application of skin rats would be high levels humidity compared to day 7 because the cream base is not absorbed into the skin of mice.

Testing the effect of clam shell shell collagen cream is done with the same treatment. Mice smeared with collagen cream as much as 50 grams of cream, applied to the back of the rats that have been shaved and then applying must be evenly distributed. Collagen cream testing was carried out for 7 days, then the data results were averaged from each formulation. The results of humidity testing data can be seen in table 5.

Humidity obtained from 4 concentrations shows the results that the collagen cream at the beginning of applying the skin of rats experiencing humidity from low then increases on Monday, then after that

| Day  | F1 10.6% | ±SD 0.907 | Mytilus viridis | 11.13% | 0.200 | 11.8% | 0.872 | 14.50% | 0.656 | 16.3% | 0.819 |
|------|----------|-----------|-----------------|-------|------|-------|-------|-------|-------|-------|-------|
| Day 2| F2 10.3% | ±SD 0.651 |                 | 13.10%| 0.513| 13.16%| 0.884| 15.83%| 0.603| 17.644| 0.404 |
| Day 3| F3 10.2% | ±SD 0.252 |                 | 14.13%| 0.503| 14.36%| 0.608| 17.1% | 0.579| 19.46%| 0.525 |
| Day 4| F4 10.05%| ±SD 0.569 |                 | 14.26%| 0.529| 15.90%| 0.651| 18.16%| 0.416| 20.16%| 0.569 |
| Day 5| F5 10.00%| ±SD 0.300 |                 | 13.20%| 0.436| 14.66%| 0.803| 16.76%| 0.531| 18.94%| 0.569 |
| Day 6| F6 10.04%| ±SD 0.200 |                 | 13.26%| 0.306| 13.3% | 0.834| 15.96%| 0.500| 18.46%| 0.453 |
| Day 7| F7 10.02%| ±SD 0.416 |                 | 12.20%| 0.385| 12.30%| 0.734| 14.93%| 0.872| 17.46%| 0.686 |

*Keterangan : Base = cream base
±SD = Standard Deviasi
F1 = Collagen contentration 10%
F2 = Collagen contentration 15%
F3 = Collagen contentration 20%
F4 = Collagen contentration 25%
there is a decrease in humidity from Tuesday to Thursday. This happens because the process of absorption of active substances in the form of collagen at the beginning of the application appears to have increased from Saturday to Sunday but the peak of skin moisture increase occurs on Monday. The process of increasing humidity is due to a cream containing collagen which has perfect absorption for more or less on initial use for 4 days. The longer the collagen cream attached to the skin of the back of the rat, the collagen will not react because the application of collagen cream in this experiment is done only once and rub changes are seen from day 1 and day 7.

The benefits of collagen in a cream as an active ingredient that functions as a moisturizing effect when tested on the back of a rat's skin. Giving creams to the skin is a way to give medicine to the skin by applying which aims to maintain the skin from hydration or dryness, protect the surface of the skin and treat moisture to the skin. The higher the collagen applied to the skin of mice, it will produce a more supple skin. From the 7-day trial test of 4 concentrations of collagen, it was found that the skin with a cream base shows that the skin is still thin and pink, after being applied with a collagen cream, the concentration of 1% occurs, changes in skin color begin to change and the skin texture begins to spring. At concentrations of 10%, 15%, 20% and 25% the change is more pronounced with the skin color getting thicker from the start and the texture of the skin more elastic according to the high concentration of collagen used in the cream.

Collagen cream testing was carried out on the back skin of mice. The choice of the back as a part of the rat used for the experiment is because the structure of the skin consists of edipermis, dermis and hypodermis. From there it can be concluded that the process of absorption of collagen active substances that occur in the skin from the skin's structural tissues occurs well because the method used is the method directly to the intended center, which is a cream against rat skin. Collagen cream begins to absorb into the skin tissue of mice, resulting in changes in skin moisture from skin types that are not so moist become more moist and skin texture is more supple. The process of absorption of collagen cream does not occur directly, but seen first for 1 day in a different time, from there it can be seen that the moisture of the skin of mice from 1 o'clock onwards changes occur. Changes that occur due to the active ingredient of collagen have an effect as a moisturizer on the skin of mice so that this study was successful (Setiadi, 2007).

3.5 Results of data analysis

| Table 6. Test of Normality |
|---------------------------|
| Formulation | Shapiro-Wilk |
|              | Df | Sig.  |
| Humidity     |     |       |
| Base cream   | 14 | 0.006 |
| Formulation 10% | 14 | 0.200 |
| Formulation 15% | 14 | 0.671 |
| Formulation 20% | 14 | 0.834 |
| Formulation 25% | 14 | 0.616 |

| Table 7. Test of Homogeneity of Variance |
|-----------------------------------------|
| Levene Statistic | df1 | df2 | Sig.  |
|------------------|-----|-----|-------|
| 5,542            | 4   | 65  | 0.002 |

**Hipotesis:**
H0 = same variance
V1 = variance not same

| Table 8 Kruskall-Wallis Test |
|------------------------------|
| Humadity | Chi square | df | Arymp. Sig. |
|----------|------------|----|-------------|
|          | 59,368     | 4  | 0,000       |

**Hipotesis:**
H0 = concentration does not affect humidity
V1 = concentration affects humidity

| Table 9 Uji Mann Whitney |
|-------------------------|
|                         | Humidity       |
| Mann-Whitney U          | 0.000          |
| Wilcoxon W              | 100.000        |
| Z                       | -2.510         |
| Asymp.Sig(2-tailed)     | 0.000          |

**Hypothesis:**
H0 = concentration doesn't affect humidity
V1 = concentration affects humidity

In table 6, it can be seen that the significant value shows the result > 0.05 which means that each of the data is normally distributed. Then the variant homogeneity test was performed. This test is used to see one of the anova assumptions, namely the existence of the same variant of the analyzed sample. Homogeneity variance test results can be seen in table 7.

In table 7, the probability significance value of 0.001 or <0.05 means that H0 is rejected or the variance is different. The results of the transformation of the data showed a significant value <0.05. Therefore, one way ANOVA test could not be performed so a non parametric test was performed using kruskal-wallis to determine the average difference of each formulation. The results of kruskal-wallis calculations can be seen in table 8.

Based on the results of the kruskal-wallis calculation in table 8, the significant value indicates the number 0.000 or p <0.05; then H0 is rejected H1 is accepted or it can be concluded that concentration influences humidity.

Statistical analysis was continued with the Mann Whitney test to determine significant differences between humidity levels. The results can be seen in table 9.

The results of the mann whitney test are known that the Asymp value. Sig. (2-tailed) of 0.000 or p <0.05. It can be concluded that H1 is accepted or concentration influences humidity.

4. Conclusions and Recommendations
4.1 Conclusions
Based on the results of research that has been done, it can be concluded that blasting cream Anadara granosa and Mytilus viridis can provide a moisturizing effect on the skin of rat. The most optimal concentration as a moisturizing effect on rat skin is a concentration of 25%.

4.2 Recommendations
Suggestions that can be given for further research are further research needs to be done to find out what content is contained in the shells of blood clams and green shells. It is necessary to experiment using a rabbit test animal to determine the effect of humidity with a comparison of male rat test animals.

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