Anti-Aging Effectiveness of Avocado Peel Extract Ointment (*Persea americana* Mill.) against Hydration, Collagen, and Elasticity Levels in Wistar Rat

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Authors’ contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The skin is a complex organ that protects the body from the external environment. The skin has a variety of functions such as providing a physical permeability barrier, protection from infectious agents, thermoregulation, sensation, protection against ultraviolet (UV) rays, regeneration, and wound healing. Clinically, aging skin is characterized by loss of hydration, rough texture, irregular pigmentation, yellowish discoloration, telangiectasia, deep wrinkles or wrinkles, thinning of the skin, and fine lines. One of the plants that can be used as natural ingredients that are rich in antioxidants and interesting to study is avocado (*Persea americana* Mill.) from the Lauraceae family, which is a plant that has medicinal properties and contains a variety of nutrients. Besides being consumed as food, avocados are also used as a mixture of cosmetic products. This study aims to assess the
anti-aging effectiveness of avocado peel extract on increasing collagen levels, elasticity, and hydration in male rats. A total of 25 rats were divided into 5 groups, each group consisting of 5 white rats; control group (ointment base), avocado peel extract ointment group 2.5%, 5%, 7.5%, and 10%. The entire group of rats will be measured the condition before treatment with the Skin Analyzer EH 900 U including levels of collagen, elasticity, and hydration. After measuring the initial skin condition, the treatment was started by applying ointment until it was evenly distributed over the marked area, the ointment was applied according to the groups that have been set above, the application was carried out 2 times a day for 4 weeks. Changes in skin condition were measured weekly for 4 weeks using the EH 900 U skin analyzer. The results of this study indicate that the methanol extract of avocado peel contains phytochemicals in the form of alkaloids, tannins, phenols, flavonoids, and glycosides. Then the phenolic, flavonoid, and tannin content of the avocado peel methanol extract was 59.55 GAE mg/gram extract, 2.96 QE mg/gram extract, and 22.63 TAE mg/gram extract. After 4 weeks of treatment, there was a significant change in the level of hydration, collagen, and skin elasticity (p<0.05) in all treatment groups, this can be seen from the P-value <0.05, with the group of rats receiving the highest concentration of ointment (10%) showed the highest average, namely hydration 49.60%, collagen 40.20%, and elasticity 68.20%. Changes in hydration levels, collagen, and skin elasticity before and after treatment also showed significant changes (P-value < 0.05) with the highest changes found at a concentration of 10%, namely hydration 88.40%, collagen 60.0%, and elasticity 68.20%. In conclusion, the results of this study showed that administration of avocado peel extract ointment could increase hydration, collagen, and skin elasticity levels compared to controls groups, positively correlated with the increase of the duration and the quantity of avocado peel extract ointment were given. The best formulation that gives the most significant effect is 10% avocado peel extract ointment by increasing levels of hydration, collagen, and elasticity the most significantly.

Keywords: Anti-aging; collagen; elasticity; hydration; avocado peel.

1. INTRODUCTION

The skin has various functions such as providing a physical permeability barrier, protection from infectious agents, thermoregulation, sensation, protection against ultraviolet (UV) rays, regeneration and wound healing [1]. Clinically, aging skin is characterized by loss of hydration, rough texture, irregular pigmentation, yellowish discoloration, telangiectasia, deep wrinkles or wrinkles, thinning of the skin, and fine lines. The existing explanation for the emergence of this feature is that radiation increases reactive oxygen species (ROS), which at higher concentrations can cause damage to DNA molecules, fatty acids, carbohydrates, and proteins, including collagen and elastin. In general, aging skin exhibits an imbalance between adequate loss and replenishment of structural and functional components. The skin fiber skeleton is one of the structures most severely affected by aging, and its degeneration leads to a gradual loss of tissue support with consequent aesthetic impact [2].

Under normal conditions, the skin produces enzymes such as elastase and collagenase. Reactive Oxygen Species (ROS) factor or excessive exposure to UV light will accelerate the activation process of the elastase enzyme, which is the only enzyme capable of degrading elastin. Elastin is a major component of the elastic fibers of connective tissue and tendons. The elastin fibers in the skin, together with the collagen fibers, make up the subcutaneous tissue of the epidermis. Due to the activation of these enzymes, it destroys all the major connective tissue matrix proteins of the skin, including elastin, collagen, and proteoglycans, where collagen is responsible for the tensile strength of the dermal matrix, elastin is responsible for its elasticity, and proteoglycans provide viscosity and hydration to the skin, skin, resulting in disruption of these functions. Due to the importance of the main connective tissue matrix components of the skin, such as collagen, elastin, and proteoglycans, they have been the subject of most anti-aging studies on the skin [3,4].

As the body's most volume organ, the skin shows the most obvious signs of aging as a person gets older. Therefore, for many people, especially women, a large amount of daily expenditure is spent on cosmetics and medicines that seek to prevent or reverse skin aging. This broad cosmetic need continues to promote research on skin aging and its treatment with
various creams that claim to have anti-aging effects [4]. Collagen content is the most commonly used anti-aging parameter [5]. In addition, anti-aging parameters that are often used are elasticity and hydration levels [4].

In modern times, many antioxidant agents are used either as supplements or topical creams, but synthetic antioxidant agents are limited because of their side effects, synthetic antioxidants are also more likely to cause irritation or allergies to the skin. Therefore, many studies have been conducted to find natural antioxidants from herbal plants that have antioxidant activity so that they can prevent side effects and allergic reactions [6]. Indonesia is a country that has natural wealth with various types of plants that can be efficacious as traditional medicine and have various good ingredients, such as antioxidants. Natural medicines derived from plants are increasingly in demand by the public because the vegetable ingredients are easy to obtain, easy to mix and the price is also more affordable [7].

One of the plants that can be used as natural ingredients that are rich in antioxidants and interesting to study is avocado (Persea americana Mill.) from the Lauraceae family, which is a plant that has medicinal properties, this plant can grow in tropical and subtropical areas. Avocado is a fruit that is very popular with the public because it tastes delicious and contains a variety of nutrients. Besides being consumed as food, avocados are also used as a mixture of cosmetic products. However, the use of so many avocados is not accompanied by the use of seeds and peel. So far, avocado peel and seeds tend to be thrown away. Avocados are one of the medicinal plants that are known to have antioxidant properties because they contain antioxidant compounds such as saponins, alkaloids, and flavonoids in the fruit, seeds, and leaves. In addition, the leaves also contain polyphenols, and the fruit contains tannins [8,9].

According to research by Rotta et al. [10], avocado peel extract in tea preparations contains antioxidant compounds in the form of phenols and flavonoids which are higher than apple tea [11]. In Aminah et al's research (2017), avocado peel extract contains high levels of flavonoid compounds where flavonoids have antioxidant, antibacterial, antiviral, anti-inflammatory, anti-allergic, and anticancer activities [7]. According to the phytochemical test in the research of Wulandari et al. [8], avocado peel extract contains alkaloids, flavonoids, saponins, tannins, polyphenols, steroids, and triterpenoids compounds. Phenol and flavonoid compounds can reduce the process of formation and binding of free radicals in the human body [11]. Saponin compounds can also stimulate the formation of collagen by fibroblasts [12]. The use of thick extracts on the skin is not practical and not optimal, so it is necessary to make preparations that can stick to the skin for a long time, namely semisolid preparations in the form of ointments. The ointment is a semisolid preparation that is soft, easy to apply, easy to clean, and is often used as an external drug on the skin and mucous membranes [13]. The base of the ointment is usually vaseline, but it can also be lanolin or oil. Vaseline has an emollient, occlusive effect, and can stay on the skin surface for a long time without drying out. The dominant oil properties in vaseline can maintain skin moisture and prolong drug contact with the skin and can increase the absorption of the drug’s active substance [14,15].

Research using a skin analyzer has also been carried out quite a lot, one of which is Hanum & Lalia's (2018) research on the anti-aging and anti-acne effects of andaliman ethanol extract peel off masks by examining moisture content, smoothness, pore size, black spots, and skin wrinkles [16]. In addition, Choi et al (2016) also researched the protective effect of apigenin on aging skin using a cream containing apigenin by examining the levels of elasticity, wrinkles, smoothness, and skin moisture [17]. Then Prasetyo et al. [18] also researched physical evaluation and anti-aging effects of peel-off gel masks from red bean extract by observing the increase in moisture content, pore size, fineness, and the number of dark spots using a skin analyzer.

Based on the description of the background above, the researcher is interested in researching the anti-aging activity test of avocado peel extract because it is suspected that there is an anti-aging activity from avocado peel extract because it contains various antioxidants.d justification of the work done [1,2].

2. METHODS

This type of research is non-experimental and experimental, using a pre-test post-test control group design. Non-experimental research includes determination of avocado peel,
extraction, and preparation of anti-aging ointments using avocado peel extract with concentrations of 2.5%, 5%, 7.5%, and 10%. Experimental research includes testing the anti-aging activity of avocado peel extract.

This research was conducted at the Department of Pharmacology, Faculty of Medicine, Indonesia Prima University, in December-February and has been approved by Health Research Ethics Committee from Universitas Prima Indonesia with registration no. 007/KEPK/UNPRI/XI/2021.

2.1 Materials

Avocado peel, distilled water, hard paraffin, lanolin, white vaseline, filter paper, Cetostearyl alcohol, wax paper, ethanol 96%, FeCl3, NaOH 10%, HCl 2N, sulfuric acid, chloroform, Mayer's reagent, dragendroff's reagent.

2.2 Instrument

Rotary evaporator, water bath, macerator, ointment container, knife, stirring rod, skin analyzer EH-900U, shaver, oil paper, filter paper, test tube.

2.3 Plant Collection and Identification

The research sample, namely avocado, was obtained from Brastagi, North Sumatra, and identified at the Medanese Herbarium (MEDA), Plant Taxonomy Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, University of North Sumatra, Medan.

2.4 Extraction

The avocado peel is cut into small pieces and dried for a few days. After drying, put into a maceration vessel. In the vessel filled with ethanol 96% with a ratio of 1:7.5 (simplicia: solvent). Furthermore, the vessel is closed and allowed to stand for 5 days in a place protected from the sun and damp, stirring occasionally. When it's been 5 days then filtered and extracted the dregs again, done 1 time to get more extract. Then the extract is concentrated with a rotary evaporator and evaporated through heating until the extract is dry [19].

2.5 Qualitative Examination of Alkaloid

2 mL of the test solution was evaporated on a porcelain dish until a residue was obtained, then dissolved with 5 mL of 2N HCl. Once cool, the solution is filtered. The solution obtained is divided into 3 test tubes. The first tube serves as a control. The second tube was added 3 drops of dragendroff reagent and the third tube was added 3 drops of Mayer reagent (through the tube wall). The formation of orange deposits in the second tube and yellow deposits in the third tube indicates the presence of alkaloids [20].

2.6 Qualitative Examination of Saponin

4 mL of the test solution are added to 5 mL of distilled water, shake, see that there is a stable foam. A little extract is added to 5 mL of water, shake it in a test tube, a stable foam is formed (1 cm high foam and stable for 30 minutes). 4 mL of the test solution was put into the test tube as a control [21].

2.7 Qualitative Examination of Flavonoid

1 mL of each test solution is inserted into 3 test tubes. Tube 1 as a control, tube 2 added with 1 mL of 5% FeCl3 solution, positive for flavonoids if there is a dark green/blue color change. Tube 3 added with a few drops of 10% NaOH forms a yellow color if it contains flavonoids [22].

2.8 Qualitative Examination of Triterpenoid

2 mL of aqueous plant extract, 2 mL of chloroform are added and this is followed by the addition of a few drops of concentrated sulfuric acid. The solution is well shaken. The formation of a yellow undercoat indicates the presence of terpenoids [23].

2.9 Qualitative Examination of Tannin

2 mL of the test solution is put into a test tube added with a few drops of 1% FeCl3 solution, a positive sign of tannin if the color formed is dark green/blue [24].

2.10 Qualitative Examination of Phenols

The test solution was added with FeCl3 (1% in water/ethanol) in a drip plate, a positive sign of phenols if there was a green/red/purple/blue/black color change [24].
2.11 Determination of Total Flavonoid Content

A total of 1 ml of sample was added to 1 ml of 50% ethanol, then added 0.1 ml of 10% AlCl3 solution. After being incubated for 30 minutes, the absorbance readings were carried out at the maximum wavelength. Determination of total flavonoid levels is determined by the following formula [25]:

\[
\text{TFC} = \frac{\text{Quercetin Equivalent } \times \text{ Extract Solvent Volume}}{\text{Mass of Extract}}
\]

\[
= \frac{\text{Quercetin Equivalent}}{\text{Concentration}}
\]

2.12 Determination of Total Tannin Content

1 ml of sample solution and put into a 10 ml measuring flask, add 0.5 ml of folic denise reagent and 1 ml of saturated sodium carbonate solution (35%) (Na2CO3), then add aqua dest to 10 ml. Aquadest was used as a blank as a substitute for the sample. Tannic acid is used as a standard at various concentrations. Total tannin levels are expressed in units of mg of tannic acid equivalent / g sample (mg TAE / g). The determination of total tannin content is determined by the following formula [26]:

\[
\text{TTC} = \frac{\text{Tannic Acid Equivalent } \times \text{ Volume of extract solvent}}{\text{Mass of Extract}}
\]

\[
= \frac{\text{Tannic Acid Equivalent}}{\text{Concentration}}
\]

2.13 Determination of Total Phenol Content

0.1 mL of the extract was added with 0.5 mL of the Folin-Ciocalteu reagent. Stir the solution and let stand for 6 minutes. Add 2.5 ml of the 5% sodium carbonate solution. Then the mixture was incubated for 30 minutes at room temperature. The absorbance readings were carried out at the maximum wavelength. Aquadest was used as a blank as a substitute for the sample. Gallic acid is used as a standard at various concentrations. Phenolic levels are expressed in units of mg equivalent of gallic acid / g sample (mg GAE / g). Determination of total phenolic levels is determined by the following formula [27]:

\[
\text{TPC} = \frac{\text{Gallic Acid Equivalence } \times \text{ Volume of Extract Solvent}}{\text{Mass of Extract}}
\]

\[
= \frac{\text{Gallic Acid Equivalence}}{\text{Concentration}}
\]

2.14 Formulation of Ointments

The various materials used are heated according to the melting point of each ingredient. The formulations were made with various concentrations as shown in Table 1 [28].

2.15 Intervention

Anti-aging activity testing using 25 male rat samples and divided into 5 groups. Group 1 was treated by using F0 (control), group 2 was treated by using F1 (2.5% avocado peel extract ointment), group 3 was treated by using F2 (5% avocado peel extract ointment), group 4. Treated by using F3 (7.5% avocado peel extract ointment), and group 5 treated by F4 (10% avocado peel extract ointment).

The rats were placed in a cage with a size of 40 cm x 20 cm x 10 cm, with a temperature of 25-27oC, with wood husks that were replaced every two days. Light came from the window during the day, cross ventilation at the top of the cage. 1 cage filled with 5 rats. All rats have acclimatized for 7 days with the aim that the test animals were able to adapt to the environment that would be occupied during the study.

Table 1. Topical dosage formulations for each ointment

| Ingredient          | Ointment Base | 2.5% Avocado Peel Ointment | 5% Avocado Peel Ointment | 7.5% Avocado Peel Ointment | 10% Avocado Peel Ointment |
|---------------------|---------------|---------------------------|--------------------------|---------------------------|--------------------------|
| Avocado Peel Extract| -             | 0.25 ml                   | 0.5 ml                   | 0.75 ml                   | 1 ml                     |
| Lanolin             | 2.5g          | 2.5g                      | 2.5g                     | 2.5g                      | 2.5g                     |
| Hard Paraffin       | 2.5g          | 2.5g                      | 2.5g                     | 2.5g                      | 2.5g                     |
| Cetostearyl alcohol | 2.5g          | 2.5g                      | 2.5g                     | 2.5g                      | 2.5g                     |
| White Vaseline      | 42.5g         | 42.5g                     | 42.5g                    | 42.5g                     | 42.5g                    |
The entire group of rats will be shaved on the back of an area of 2x2 cm² using a manual hair shaver. Then measured the condition of the test animals before treatment with the Skin Analyzer EH 900 U including levels of collagen, elasticity, and hydration. After measuring the initial skin condition, the treatment was started by applying cream until it was evenly distributed over the marked area, the cream was applied according to the groups that have been set above, the application was carried out 2 times a day for 4 weeks. Changes in skin condition were measured weekly for 4 weeks using the EH 900 U skin analyzer.

2.16 Data Analysis

Data analysis in this study was carried out using IMB SPSS 25 software. Data on collagen, elasticity and skin hydration were statistically analyzed using descriptive statistical analysis by assessing the central tendency in the form of mean, median, and mode, followed by the value of data distribution (dispersion). Then statistical analysis continued with normality test with Shapiro-Wilk test. If the data is normally distributed, statistical analysis is continued with the One Way Anova test and the Post Hoc Test. However, if the data in this study is not normally distributed, then the data will be transformed so that the data is normally distributed. If the data remains abnormal, use the Kruskal-Wallis Test.

3. RESULTS

3.1 Identification of Sample

This study was used the avocado peel from a traditional market in the Medan, North Sumatera. Before the sample was extracted, the sample was identified in the Herbarium Medanese (MEDA) at the University of North Sumatra. The result of identification was shown as the following.

Kingdom : Plantae
Division : Spermatophyta
Class : Dicotyledoneae
Ordo : Laurales
Familia : Lauraceae
Genus : Persea
Species : Persea americana Mill.

3.2 Phytochemical Screening of Avocado Peel Methanol Extract

Based on the results of phytochemical screening on avocado peel methanol extract conducted at the organic chemistry laboratory of FMIPA, University of North Sumatra, it was found that the avocado peel methanol extract contains chemical compounds, which can be seen in Table 2.

The phytochemical screening showed that the avocado peel extract contained alkaloids, flavonoids, phenols, tannins, and glycosides.

| Phytochemical                  | Methods                                  | Interpretation |
|-------------------------------|------------------------------------------|----------------|
| Alkaloid                       | Bouchardart                              | +              |
|                               | Maeyer                                   | -              |
|                               | Wagner                                   | -              |
|                               | Dragendorff                              | +              |
| Triterpenoid and Steroid       | Salkowsky                                | -              |
|                               | Lieberman-Burchard                       | -              |
| Saponin                       | Aquadest + Alcohol 96%                   | -              |
| Flavonoid                     | FeCl₃ 5%                                 | +              |
|                               | Mg(1) + HCl (p)                          | +              |
|                               | NaOH 10% (p)                             | -              |
|                               | H₂SO₄(p)                                 | -              |
| Phenol                        | FeCl₃ 1%                                 | +              |
| Tannin                        | FeCl₃ 1%                                 | +              |
| Glycoside                     | Water + NaOH 10%                         | +              |
3.3 Determination of Total Levels of Phenols, Flavonoids, and Tannins

Furthermore, the phytochemical analysis was continued to determine the total phenol, tannin, and flavonoid content. The result of this analysis was shown by the following Table 3.

Based on Table 3, the total phenol, tannin, and flavonoid from methanol extract of avocado peel respectively contained 59.55 GAE mg/gram extract, 22.63 TAE mg/gram extract, and 2.96 QE mg/gram extract.

3.4 Evaluation of Anti Aging Activity

In the evaluation of the anti-aging activity of the avocado peel methanol extract ointment, it was assessed through 3 parameters including hydration, collagen, and elasticity.

3.4.1 Hydration

The level of skin hydration at all groups of rats can be seen in Table 4. From the Table 4 data below, it can be seen that the level of skin hydration of all treatment groups at the beginning of the study was uniform, this can be seen from the P-value > 0.05 (P-value = 0.559). After 4 weeks of treatment, there was a significant change in the level of skin hydration in all treatment groups, this can be seen from the P-value < 0.05. After 4 weeks of treatment, the group of rats that received the highest concentration of ointment showed the highest average level of skin hydration, which was 49.60%, followed by a lower concentration of 7.5% (46.20%), 5% (38.60), and 2.5% (38.00%). Changes in the level of skin hydration before and after treatment were described by the percentage change in skin hydration which also showed significant changes (P-value < 0.05) with the highest change found at a concentration of 10%, namely 88.40% and the lowest in the control group, namely 43.34%.

3.4.2 Collagen

The description of skin collagen levels in all groups of rats can be seen in Table 5.

Table 3. Total of phenol, tannin, and flavonoid content

| Phytochemical                        | Phytochemical Level |
|--------------------------------------|---------------------|
| Phenol (GAE mg/gram extract)         | 59.55 ± 0.39        |
| Tannin (TAE mg/gram extract)         | 22.63 ± 0.00        |
| Flavonoid (QE mg/gram extract)       | 2.96 ± 0.42         |

GAE: Gallic Acid Equivalent; QE: Quersetin Equivalent; TAE: Tannic Acid Equivalent

Table 4. Comparison of hydration levels in all treatment groups

| Group                     | Hydration Level | Percentage Change in Hydration |
|---------------------------|-----------------|--------------------------------|
|                           | Initial#        | Final##                        |                                  |
| Control                   | 25.00 ± 1.58    | 31.40 ± 1.52^a                 | 25.38 ± 6.92^a                   |
| Avocado Peel extract ointment | 49.60 ± 2.51^c  | 88.40 ± 10.78^c                | 88.40 ± 10.78^c                  |
| 7.5%                      | 46.20 ± 2.28^c  | 79.72 ± 16.75^c                | 79.72 ± 16.75^c                  |
| 5%                        | 38.60 ± 1.14^cd | 52.31 ± 10.02^d                | 52.31 ± 10.02^d                  |
| 2.5%                      | 38.00 ± 1.00^d  | 43.34 ± 7.76^bc                | 43.34 ± 7.76^bc                  |

P-Value: 0.559 < 0.05^* < 0.05^**

*All data are presented as Mean ± SD; *Different superscripts in the same column showed significant differences and P values were obtained through One Way ANOVA and Post Hic Test Tukey HSD

Table 5. Comparison of collagen levels in all treatment groups

| Groups                      | Collagen | Percentage change in Collagen |
|-----------------------------|----------|-------------------------------|
|                             | Initial## | Final##                       |                                  |
| Control                     | 25 (24-26)| 31.60 ± 1.14^a                | 25.00 (23.08-33.33)^a            |
| Avocado Peel Methanol       | 10%      | 25 (24-26)                    | 40.20 ± 0.84^b                   | 60.00 (57.69-62.50)^c           |
| Extract Ointment            | 7.5%     | 25 (24-26)                    | 38.40 ± 0.55^c                   | 56.00 (46.15-58.33)^cc           |
| 5%                          | 25 (24-26)| 36.40 ± 0.55^d                | 48.00 (42.31-56.52)^c            |
| 2.5%                        | 24 (23-27)| 34.40 ± 0.55^e                | 41.67 (29.63-47.83)^ac           | 0.913^< 0.05**                  | 0.001^*                        |

# Data is displayed as Mean ± SD; ## Data is displayed as Median (Min-Max); *Different superscripts in the same column showed significant differences and P values were obtained through One Way ANOVA and Post Hic Test Tukey HSD; **Different superscripts in the same column show significant differences and P values obtained through Kruskal-Wallis
From the table data above, it can be seen that the level of skin collagen from all treatment groups at the beginning of the study was uniform, this can be seen from the P-value > 0.05 (P-value = 0.913). After 4 weeks of treatment, there was a significant change in skin collagen in all treatment groups, this can be seen from the P-value < 0.05. After 4 weeks of treatment, the group of rats that received the highest concentration of ointment showed the highest average skin collagen level of 40.20%, followed by a lower concentration of 7.5% (38.40%), 5% (36.40%), and 2.5% (34.40%). Changes in the level of skin collagen before and after treatment were described by the percentage of changes in skin collagen which also showed significant changes (P-value = 0.001) with the highest change found at a concentration of 10%, namely 60.00% and the lowest in the control group, namely 25.00%.

3.4.3 Elasticity

The description of skin elasticity levels in all groups of rats can be seen in Table 6.

From the Table 6 data below, it can be seen that the level of skin elasticity of all treatment groups at the beginning of the study was uniform, this can be seen from the P-value > 0.05 (P-value = 0.900). After 4 weeks of treatment, there was a significant change in skin elasticity in all treatment groups, this can be seen from the P-value < 0.05. After 4 weeks of treatment, the group of rats that received the highest concentration of ointment showed the highest average skin elasticity level of 68.20%, followed by a lower concentration of 7.5% (61.20%), 5% (58.40%), and 2.5% (57.00%). Changes in the level of skin elasticity before and after treatment were described by the percentage change in skin elasticity which also showed a significant change (P-value < 0.05) with the highest change found at a concentration of 10%, namely 44.63% and the lowest in the control group, namely 8.33%.

4. DISCUSSION

The results of this study indicate that the methanol extract of avocado peel contains phytochemicals in the form of alkaloids, tannins, phenols, flavonoids, and glycosides. Then according to Rochma [30], avocado peel extract contains phytochemicals in the form of flavonoids and alkaloids. In addition, according to the phytochemical test in Putri's research [31], avocado peel extract contains alkaloids and flavonoids.

Table 6. Comparison of elasticity levels in all treatment groups

| Groups                        | Elastivity | Percentage change in Elasticity* |
|-------------------------------|------------|----------------------------------|
|                               | Initial#   | Finalf                           |
| Control                       | 47 (46-48) | 48.80 ± 4.09^a                    | 8.33 ± 3.59^a |
| Avocado Peel Methanol Extract | 47 (46-48) | 68.20 ± 1.48^b                    | 44.63 ± 5.18^b |
| Ointment 10%                  | 46 (46-48) | 61.20 ± 1.30^c                    | 32.60 ± 4.54^c |
| 5%                            | 47 (46-48) | 58.40 ± 1.14^cd                   | 27.68 ± 5.58^d |
| 2.5%                          | 47 (46-48) | 57.00 ± 1.58^d                    | 18.82 ± 3.96^d |
| P-Value                       | 0.900      | < 0.05*                           | < 0.05*        |

# Data is displayed as Mean ± SD; # Data is displayed as Median (Min-Max); *Different superscripts in the same column showed significant differences and P values were obtained through One Way ANOVA and Post Hic Test Tukey HSD.
This study stated that there was an increase in hydration, collagen, and elasticity levels after treatment by applying avocado peel extract ointment. According to various studies, the avocado peel contains various phytochemicals that can act as anti-aging agents. According to research by Vinha et al. [34], the avocado peel contains phenols, flavonoids, carotenoids, vitamin C, and vitamin E. According to research by Kim et al. [35], phenolic compounds can increase collagen synthesis by accelerating the formation of fibrils. According to research by Leheta et al. [36], deep peeling using phenol is effective for treating post-acne atrophic scars. Collagen is the most abundant component of the extracellular matrix, a protein that determines skin physiology by maintaining skin structure and enabling its various functions. The extracellular matrix retains water and supports smooth and firm skin. Oral administration of collagen supplements can also improve skin hydration, elasticity, and smoothness [37].

This study is in accordance with the theory that polyphenols increase the proliferation and production of collagen in fibroblasts. To minimize the formation of wrinkles, stimulation of collagen synthesis as well as renewal of damaged collagen fibers is essential. Restoration can be achieved due to MMP inhibition which modulates the expression and production of matrix metalloproteinases via activation of AP-1 and NF-B. Tyrosine which is also one of the phenolic compounds can suppress the synthesis of melanin. Phenols and flavonoids have a structure similar to tyrosine, therefore phenols and flavonoids can also act as inhibitors of substrate analogs against melanogenesis [38]. This study is in accordance with the theory of Anggraini et al. [39], that the flavonoid, phenol, and terpenoid components inhibit the production of elastase, an enzyme that degrades elastin, so that skin elasticity is maintained.

This study also agrees with the theory that vitamin E (α-tocopherol) used as a component of skin products has anti-inflammatory and antiproliferative effects in concentrations between 2 and 20%. It works by smoothing the skin and increasing the ability of the stratum corneum to retain moisture, accelerating epithelialization, and contributing to skin protection [40]. Moisturizing agents can be categorized into several groups, namely occlusive moisturizers, humectants, and intercellular lipids in the stratum corneum (SC). Occlusive and humectant ingredients are the most formulated ingredients in moisturizing product components because they have a mixture of fats that can restore skin moisture. The mechanism of the moisturizer to hydrate the skin is to reduce transepidermal water loss (TEWL) and attract water to hydrate the SC and epidermis. Some materials that can reduce the occurrence of occlusive TEWL include petroleum, paraffin, dimethicone, cyclo-methicone, and mineral oil [41].

The results of this study are in line with the research of Silitonga [42], where avocado peel extract in a peel-off gel mask preparation affects the anti-aging effect with the highest concentration showing an increase of 7.5% where at this concentration it shows an increase in skin condition for the better, including increased skin hydration levels, smoother skin, smaller pores, reduced number of blemishes and the most significant reduction in the number of wrinkles during four weeks of treatment. Efriana's research [43] also states that avocado peel extract made in sheet mask preparations can also increase skin hydration. According to Fabitiary's research [44], the use of avocado peel masks can increase skin moisture.

5. CONCLUSION

This study indicates the administration of avocado peel extract ointment increased the average of hydration, collagen, and elasticity level compared to the control group, positively correlated with the increase of the duration and the quantity of avocado peel extract ointment were given. The best formulation that gives the most significant effect is 10% avocado peel extract ointment by increasing levels of hydration, collagen, and elasticity the most significantly.

CONSENT

It is not applicable

ETHICAL APPROVAL

All experiments have been approved by Health Research Ethics Committee from Universitas Prima Indonesia with registration no. 007/KEPK/UNPRI/XI/2021.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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