ANTIOXIDANT ACTIVITY AND EFFECTIVENESS TEST OF METHANOL EXTRACT SAMBILOTO LEAVES (*Andrographis paniculata*) ON WISTAR RATS (*Rattus novergicus*) EXPOSED TO ULTRA VIOLET RAYS

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ABSTRACT

Antioxidants can stop the damaging actions of free radicals. Antioxidants are divided into two, namely enzymatic and non-enzymatic antioxidants.

This study aims to test the effectiveness of antioxidants and anti-aging of methanol extract of sambiloto leaves (*Andrographis paniculata*) in preventing skin aging in Wistar rats exposed to ultraviolet light. This study is an experimental study using pre-post test control group design. In this study, 24 male Wistar rats were used as samples which were divided into 4 groups.

The results of the study showed that the active substances contained in the methanol extract of sambiloto leaves (*Andrographis paniculata*) tested through phytochemical testing contained secondary metabolites such as alkaloids, flavonoids, saponins, and tannins. The results of the antioxidant test of the methanol extract of sambiloto leaves (*Andrographis paniculata*) through the DPPH test obtained an IC_{50} value of 53.65. So the antioxidant test of the sambiloto leaf extract (*Andrographis Paniculata*) showed that there was an antioxidant content in the sambiloto leaf extract (*Andrographis paniculata*) with a strong category. Seeing the antiaging activity of the methanol extract of sambiloto leaves (*Andrographis Paniculata*) with the best collagen density area were groups P3 and P2, namely the treatment groups given methanol extract of sambiloto leaves (*Andrographis paniculata*) concentrations of 5% and 10% because the collagen density was already very large and dense compared to treatment group 1 and the control group in skin tissue histopathology. These results indicate that the methanol extract of sambiloto leaves (*Andrographis paniculata*) has the potential as a natural antioxidant and anti-aging agent for skin exposed to UV rays.

Keywords: Antioxidants, Methanol Extract of Sambiloto Leaves, UV Rays.

INTRODUCTION

Cellular aging can occur due to exposure and accumulation of reactive oxygen species (ROS) from UV rays, cigarette smoke, pollutants, and chemicals in cosmetic products. Exposure to ROS sources can accelerate cellular aging, for example in skin cells. Skin cell aging is characterized by facial wrinkles, dull skin color, skin thickening, gradual decrease in skin elasticity, slowing of epidermal turnover which causes a decrease in a person's aesthetics and appearance (Papaccio et al., 2022).

The skin aging process can be divided into intrinsic and extrinsic processes. Intrinsic or chronological skin aging is an unavoidable process of chronological and physiological changes. Intrinsic factors that drive skin aging are time, genetic factors and hormones. This condition is also an oxidative process associated with a progressive and age-related decrease in antioxidant capacity and an increase in the production of reactive oxygen species (ROS). Clinical signs associated with intrinsic skin aging are fine lines, xerosis (dry skin) and weakness (Krutmann et al., 2021).

Antioxidants are produced by the human body, but they are not strong enough to combat the free radicals the body produces every day (Bai H, 2020). Free radicals are atoms that contain one or more unpaired electrons that are highly reactive and unstable. Free radicals tend to take electrons from other molecules that cause abnormalities in other molecules. In the human body, free radicals will form a chain reaction and produce new free radicals that eventually increase in number. Free radicals cause degenerative diseases such as cancer, diabetes mellitus and Alzheimer's (Hamouda, 2022).

Antioxidants are substances that can slow or stop lipid oxidation. Synthetic antioxidants are commonly used in industry because they are very affordable and effective in preventing oxidation in food. Synthetic antioxidants are carcinogenic, meaning they have the potential to cause cancer. Because of these side effects, synthetic antioxidants have been banned in some countries. Natural antioxidants derived from plant extracts are considered safer than synthetic antioxidants. Some natural antioxidants obtained from food are spices, herbs, vegetables, and fruits.

Enzymatic antioxidants are important as part of the cell's defense mechanism against free radical formation and in the prevention and repair of molecular damage caused by free radicals in various situations. In physiological and pathophysiological conditions, various enzymes are responsible for modulating the redox balance. These enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and others (Lee & Park, 2021). As much as 80% of extrinsic skin aging or also called photoaging is caused by exposure to UV rays, especially excessive UV-A, where there is a decrease in the amount of collagen and elastin fibers in the skin. This can ultimately result in a rough skin surface, uneven skin tone, sagging or reduced elasticity, and wrinkles. Another cause of skin aging is a person's age, because as age increases, the skin's ability to regenerate decreases. Genetic and hormonal factors also influence the aging process. Skin damage due to photoaging is mediated by the formation of reactive oxygen species (ROS) through oxidative stress conditions, when the amount of ROS exceeds the antioxidant defense capacity in skin cells. Antioxidant compounds have been shown to be able to reduce the dangers of free radicals, especially UV radiation, by reducing UV-induced inflammation and eliminating reactive oxygen species (ROS) that are harmful to the skin (Maeda, 2018). Long-term use of synthetic antioxidants in anti-aging products is starting to be abandoned because it causes skin irritation and is carcinogenic (Wulansari, 2018). An alternative that can be used to reduce the unwanted effects of synthetic antioxidants is to use natural ingredients derived from plants. Antioxidant compounds that have anti-aging activity are spread in several parts of plants such as wood, bark, roots, leaves, fruit, flowers, and seeds (Wiratantri, 2020). One of them comes from the leaves of the sambiloto plant.

Sambiloto is one of Indonesia's superior medicinal plants besides temulawak, mengkudu, pepper, aloe vera, and turmeric. As a superior plant, sambiloto has been studied botanically, cultivated, pharmacologically, chemically and clinically (Winarto, 2003). Pharmacologically, sambiloto leaves have analgesic, anti-inflammatory, antibacterial, antimicrobial, antimalarial, antiviral, immunostimulatory, hepatoprotective, cardiovascular, and anticancer properties. Research conducted by Sari (2020) found that administering methanol extract of sambiloto leaves can prevent a decrease in collagen levels and significantly increase skin tissue hydration in mice exposed to UV-B rays. In addition, the phenolic content such as flavonoids and saponins in sambiloto leaves (*Andrographis paniculata*) functions as a molecule that can inhibit the action of metalloproteinases which cause collagen destruction in the skin's dermis tissue. The antioxidants produced can prevent aging and improve signs of skin aging through the mechanism of ROS uptake and reduce reactive molecules in the body to reduce lipid peroxidation, as a collagenase inhibitor, protect the skin from UV radiation, and improve cell function (Ramadhian et al., 2017). Based on the background above, this study aims to investigate the antioxidant and anti-aging properties of methanol extract of sambiloto leaves (*Andrographis paniculata*) on skin collagen density and oxidative stress in Wistar rats exposed to UV light

LITERATURE REVIEW

The skin is an organ that covers the entire outer surface of the body, serves as a first-line barrier against pathogens, UV light, and chemicals, and provides a mechanical barrier against injury. The skin also regulates temperature and the amount of water released into the environment. The structure of the skin consists of three layers, the epidermis, dermis, and hypodermis, all of which vary greatly in anatomy and function (Yousef, 2017).

The skin is a barrier organ that separates the body from the external environment, one of which is UV rays which are the main cause of photoaging. Ultraviolet radiation is classified into UV-A (320-400 nm), UV-B (280-320 nm), and UV-C (200-280 nm). Wavelengths in the UV-C region cause unpleasant, but usually not serious, effects on the skin and eyes. UV-C from the sun is almost completely shielded by the earth's atmosphere while UVA and UVB rays reach the earth in amounts that can damage the skin structure (Bosch et al., 2015). UV-B is very detrimental to living organisms and causes erythema (sunburn) and is associated with an increased risk of cancer (Hamouda, 2022).

Excessive ultraviolet B (UVB) exposure causes the formation of intracellular reactive oxygen species (ROS). This causes oxidative stress and skin inflammation through the activation of mitogen-activated protein kinases and upregulation of transcription factors, such as activator protein 1 (AP-1). In addition, ROS stimulated by UVB can increase the expression of matrix metalloproteinase-1 (MMP-1) in fibroblasts, thereby triggering skin photoaging. MMP-1 degrades type 1 collagen, a major ECM component that provides structural support to the skin, and causes dermis decomposition and skin aging (Choi HJ, 2020). Therefore, anti-aging agents are needed to help prevent premature aging. One of the anti-aging agents is *Andrographis paniculata* leaf extract cream.

Sambiloto leaves contain many phytochemical compounds. Sambiloto leaves are rich in triterpenes, flavonoids, essential oils, alkaloids and amino acids. Sambiloto leaves have diverse and complex chemical content where the main groups include; terpenes (monoterpenes, sesquiterpenes, diterpenes, triterpenes, tetraterpenes), phenolic compounds (flavonoids, tannins), alkaloids, carbohydrates, vitamins, minerals and amino acids (Sabaragamuwa et al., 2018). It has been observed that sambiloto leaf preparations can stimulate fibroblast proliferation so that they can increase type I collagen production and reduce stretch mark formation and inflammatory reactions. Sambiloto leaves are also used as active compounds in skin care preparations due to their antioxidant, anti-inflammatory, anti-cellulite and anti-aging activities.

Antioxidants are a group of chemicals associated with significant health benefits. Antioxidants can stop the damaging actions of free radicals. Antioxidants are divided into two, namely enzymatic and non-enzymatic antioxidants. One of the enzymatic antioxidants that plays an important role in the first line of defense is superoxide dismutase (SOD). SOD catalyzes the disproportionation reaction of superoxide anion into hydrogen peroxide and molecular oxygen. Other important enzymatic antioxidants in the first line of defense include catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and peroxiredoxins (Prxs). These enzymes neutralize hydrogen peroxide, producing water and molecular oxygen (Mohania, 2017). Non-enzymatic antioxidants including ascorbate, carotenoids, flavonoids, and other phenolics to reduce the adverse effects of oxidative damage. Antioxidants are found exogenously in whole plant foods such as fruits, vegetables, or nuts and supplements (Andersen, 2018).

Sambiloto is traditionally used as a medicine for diabetes, fever, typhoid, itchy skin, snakebite medicine, antirheumatic, jaundice and pregnancy-dissolving medicine. Other properties are as a medicine for dysentery, diarrhea, acute kidney inflammation, inflammation around the ear, nose and throat (ENT), pneumonia and respiratory tract, influenza, leprosy, high blood pressure, king's lion, diuretic, and cancer. Experimental research has proven that sambiloto has antidiabetic properties (Yulinah and Fitri, 2001), hepatoprotective and anticoagulant (Hidayat R., 2022). Andrographis paniculata Nees (*Andrographis paniculata Nees*) is classified as follows:

Kingdom : Plants
Division : Spermatophyta

Subdivision : *Angiospermae*
Class : *Dicotyledoneae*
Order : *Solanaceae*
Family : *Acanthaceae*
Genus : *Andrographis*
Species : *Andrographis paniculata*Nope

METHODS

This study is a true experimental study, with the research design used is post test only control group design, which is a type of research that only observes the control and treatment groups after being given an action. The research sample in this study was male white rats (*Rattus norvegicus*) Wistar strain weighing 160-200gr and aged 2-3 months. In this study, 6 Wistar strain rats were used for each experimental group, so that the total number of test animals in this study was 24. Ethical Clearance will be submitted to the Health Research Ethics Commission (KPEK) of Prima Indonesia University and is still in process. The figure of conceptual framework are shown in figure 1.

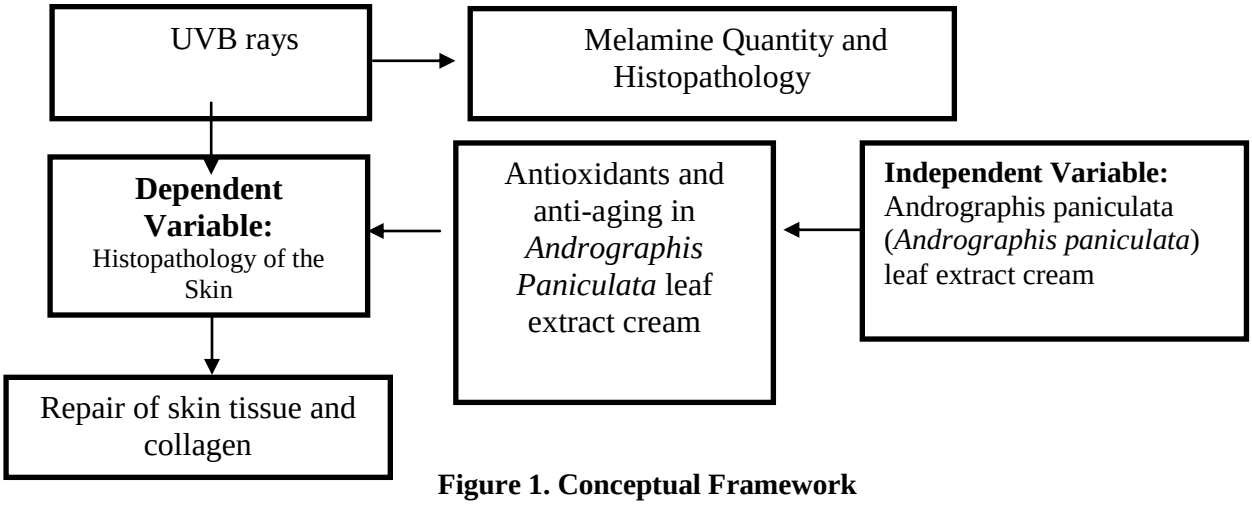


Figure 1. Conceptual Framework

The grouping of test animals was carried out randomly into 4 test groups. The following are the groups in this study:

1) Control Group (P-0) mice were exposed to ultraviolet B light and applied with base cream.
2) Treatment Group-1 (P-1), mice were exposed to ultraviolet B light and given *Andrographis paniculata* leaf extract cream with a concentration of 2.5%.
3) Treatment Group-2 (P-2), mice were exposed to ultraviolet B light and given *Andrographis paniculata* leaf extract cream with a concentration of 5%.
4) Treatment Group-3 (P-3), mice were exposed to ultraviolet B light and given *Andrographis paniculata* leaf extract cream with a concentration of 7%.

The variables used in this study arevindependent variables, dependent variables and precondition variables. The independent variablessambiloto leaf extract cream (*Andrographis paniculata*), the dependent variables are antioxidants and anti-aging, and the precondition variables are ultraviolet light B. Acclimatization is the process of adjusting to a new environment, climate, conditions, or atmosphere. Before giving treatment, all male Wistar rats went through an acclimatization process for seven days in the Laboratory of the Department of Pharmacology and Therapeutics, Faculty of Medicine, University of North Sumatra. The rats were given time to adapt to the new environment, as well as their food and drink. The provision of feed and drink to the rats was carried out according to their standard needs (ad libitum). The test animals in the form of rats were kept in groups in experimental

animal cages in the laboratory. The rat cages measuring (30 cm x 20 cm x 10 cm) were made of plastic and covered with fine wire mesh. The base of the cage was covered with 0.5–1 cm thick rice husk which were replaced every day during the study. The room lights were controlled to produce a 12-hour light/12-hour dark cycle, the temperature was set to 25–27 °C, and the room humidity was adjusted to the normal range of 35–50%.

Exposure to light was carried out with a frequency of 3 times a week (Monday, Wednesday and Friday) starting with 50 mJ/cm2 for 50 seconds in the first week, followed by 70 mJ/cm2 for 70 seconds in the second week and 80 mJ/cm2 for 80 seconds in the last week with a total UVB received of 840 mJ/cm2. Irradiation was carried out every day at 10.00 WIB using a Phillips UVB PLS9W/01/2P lamp (Haryanto et al., 2020).

Andrographis Paniculata leaf extract cream was applied after the mice were exposed to UVB light. During UVB light exposure, the extract was given twice a day for 2 weeks, 20 minutes after UVB light exposure at 09.40 WIB, 10.00 WIB and 4 hours later the administration was restarted at 14.00 WIB. Application of *Andrographis Paniculata* leaf extract cream was still given on days without irradiation.

After 2 weeks, the mice were euthanized using an overdose of ketamine (125 mg/kg BW) intramuscularly in an anaerobic jar 48 hours after the last irradiation. The skin sampling process was carried out by biopsy in the back area where the skin would be taken, cleaned of fur, the skin was cut with a thickness of approximately 2 mm to the subcutaneous with a length of 2 cm and a width of 2 cm. After that, histopathological preparations were made, and the amount of dermis collagen was calculated. The remaining unused mouse organs will be buried.

Antioxidant Test/DPPH Method

The DPPH method is an easy, fast, and sensitive method for testing the antioxidant activity of certain compounds or plant extracts. The DPPH method measures the ability of an antioxidant compound to capture free radicals. DPPH functions as a compound that acts as a free radical that will react with methanol extract as an antioxidant compound that will donate hydrogen atoms. In making the extract test solution. Weigh 10 mg of thick extract and dissolve it with methanol up to 10 mL. a solution with a concentration of 1000 ppm is obtained. Take 0.1 mL; 0.2 mL; 0.3 mL; 0.4 mL; 0.5 mL from the 1000 ppm extract solution. then add 1 ml of DPPH solution (concentration 200 ppm) to each concentration and add methanol to the mark (5 mL measuring flask). obtained concentrations of 20, 40, 60, 80, 100 ppm. Incubated for 30 minutes then the absorbance was measured using a UV-Vis spectrophotometer at a maximum wavelength of 515 nm.

The ability to capture radicals is related to the ability of a compound component to donate electrons or hydrogen. Any molecule that can donate electrons or hydrogen will react and will fade DPPH. This method is based on the color change of the DPPH radical. The color change is caused by the reaction between the DPPH free radical and one hydrogen atom released by the compound contained in the test material to form the yellow compound 1,1-diphenyl-2-picrylhydrazine. The levels of antioxidant activity are shown in table 1.

Table 1. Levels of Antioxidant Activity

Mark	Levels
IC_{50} < 50 µg/ml	Very strong
IC_{50} 50-100 µg/ml	Strong
IC_{50} 101-150 µg/ml	Currently
IC_{50} > 150 µg/ml	Weak

Antiaging Test

In this study, a sample of 24 mice and classified into 4 groups, namely control group, treatment 1, treatment 2 and treatment 3 will be exposed to ultraviolet type B (UVB) light at 15 cm. After being exposed to UVB light, each group of mice was given extraction treatment except for the control group without extraction treatment. When all treatments were completed, the researcher performed a biopsy on the back skin of the mice (2cm x 2cm x 2mm) then assessed with immunohistochemical staining to calculate MMP-1 expression and Picro Sirius Red staining to observe the density of dermis collagen (Haryanto et al., 2020). The scoring system is viewed using a light microscope with a magnification of 400x. The scoring system used is based on collagen production as follows:

Score 0 = normal
Score 1 = slight improvement
Score 2 = mild to moderate improvement
Score 3 = moderate improvement
Score 4 = moderate improvement - marked
Score 5 = marked improvement

Data from histopathological observations that have been collected, scored and then analyzed. The research data are tabulated, then the changes found are analyzed and presented descriptively. The analysis of data normality used in this study, namely the Kolmogorov-smirnov normality test. Data is normally distributed if $p > 0.05$. After conducting the data normality test, it is continued with the homogeneity test using Levene's test. Data is declared homogeneous if $p > 0.05$. Data that has gone through the normality and homogeneity tests are then processed again using the t-test to see the comparative differences between groups.

RESULTS

Based on the general characteristics of the test animals, in general the mice were in a healthy condition during this study, namely before and after treatment. A total of 24 test animals were able to participate in this study until the end without any dropouts. Body weight was measured on the 24 existing test animals. The average body weight of each group before and after treatment for 14 days was the control group 243 gr, treatment group 1 245 gr, treatment group 2 245 gr and treatment group 3 244 gr.

Preparation and Methanol Extraction of Sambiloto Leaves

Preparation of sambiloto leaves is the first stage that must be done before conducting further tests. This stage begins with separating the sambiloto leaves. The sambiloto leaves are then cut into small pieces to make it easier to dry. The drying process is carried out using an oven at a temperature of 80oC to remove the water content in the sambiloto leaves so that it does not hinder the distribution of active compounds during the maceration process. Sambiloto leaves become hard after drying due to the loss of several percent of their water content. The initial mass of fresh sambiloto leaves is 250 grams to 100 grams of dry weight. Sambiloto leaves are often ground using a blender to make powder, with the aim of increasing the surface area so that the distribution of compounds runs optimally during maceration. Sambiloto leaf powder can be seen in Figure 2.

Sambiloto leaf powder is then extracted using the maceration method. Maceration is the process of soaking samples with a suitable solvent at room temperature. The maceration method was chosen because it is a cold extraction technique that is expected to attract more antioxidant compounds (polyphenols). Polyphenol compounds are compounds that are not resistant to heat, so it is hoped that with cold extraction the compounds will not be damaged during the extraction process. The maceration process is carried out by soaking sambiloto leaves in methanol solvent for 2x24 hours. During the soaking process, stirring is carried out several times. The sample will experience the breakdown of cell walls and membranes due to the difference in pressure between inside and outside the cell during the soaking process, so

that secondary metabolites will dissolve in organic solvents. After soaking in methanol, it is then filtered, and the filtrate is taken. The sambiloto leaf filtrate is concentrated using a rotary evaporator with 100 rpm at a temperature of 65oC. The aim is to evaporate the solvent according to its boiling point. This filtrate is then used for phytochemical testing. Concentrated filtrate of methanol extract *Andrographis paniculata can be seen in Figure 2.*

Figure 2. Process of Making Dry Sambiloto Leaf Powder and Concentrated Sambiloto Leaf Methanol Extract

Antioxidant Testing

Physical antioxidant activity can be observed by the color change in DPPH. DPPH free radicals that have unpaired electrons have a purple color (Rizkayanti et al., 2017). The purple color will change to light purple or yellow when DPPH is mixed with natural compounds that can donate hydrogen atoms (Widyastuti, 2010). The higher the sample concentration, the more the purple color of DPPH fades. The DPPH solution that has been added with methanol extract of sambiloto leaves (Andrographis Paniculata) changes color from purple to faint purple. This color change can be observed in Figure 3.

Figure 3. Change in DPPH color after adding methanol extract of sambiloto leaves

The color change indicates that the methanol extract of sambiloto leaves (*Andrographis Paniculata*) has antioxidant properties. The reduction in color intensity from concentrations of 20 ppm, 40 ppm, 60 ppm, to 80 ppm indicates an increase in the antioxidant capacity of the methanol extract of sambiloto leaves in capturing DPPH radicals. This can be seen from the higher concentration of methanol extract of sambiloto leaves (*Andrographis paniculata*), the more the purple color of DPPH fades.

Antioxidant Activity Test with DPPH Method. This method is based on the color change of DPPH radicals. The color change is caused by the reaction between the DPPH free radical and one hydrogen atom released by the compound contained in the test material to form a yellow compound 1,1-diphenyl- 2-picrylhydrazine. The DPPH method is easy to use because the procedure is fast and accurate.

Table 2. Sambiloto Leaf Extract Testing (*Andrographis paniculata*) IC_{50}

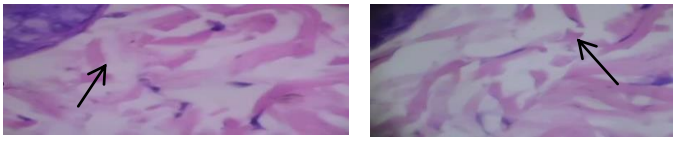
Sample name	**Concentration (ppm)**	**Percentage Damping**	**IC_{50} (ppm)**
Andrographis paniculata leaves	20	28.92	53.65
	40	39.98	
	60	59.97	
	80	85.75	

With testing at a concentration of 20 ppm, the percentage of attenuation was 28.92; at a concentration of 40 ppm, the percentage of attenuation was 39.98; at a concentration of 60 ppm, the percentage of attenuation was 28.92; at a concentration of 60 ppm, the percentage of attenuation was 59.97 and at a concentration of 100 ppm, the percentage of attenuation was 85.75. So, from all these tests, the IC_{50} value of Andrographis paniculata leaf extract was 53.65 and it can be concluded based on the concentration result category (ppm) of *Andrographis paniculata* leaf extract, it is included in the strong category. So the researcher concluded from the results of the table 2 that the antioxidants in Andrographis paniculata leaf extract are strong with a ppm concentration ranging from 50-100. Antioxidants are chemical compounds that can donate one or more electrons to free radicals, so that these free radicals can be suppressed.

Antiaging Testing

The process of taking skin samples was carried out by biopsy in the back area where the skin would be taken, cleaned of fur, the skin was cut with a thickness of approximately 2 mm to the subcutaneous with a length of 2 cm and a width of 2 cm. After that, histopathological preparations were made, and the amount of collagen and melanin was calculated as post-test data. The remaining unused mouse organs will be buried. Based on the observed data, it can be concluded that the average percentage of the best collagen density area is group P2 and P3, namely the treatment group given sambiloto leaf extract cream. with a concentration of 5% and 10%, Then the average percentage of the worst collagen density area is the control group, namely the group that was only given ultraviolet b light exposure treatment but was not given sambiloto leaf cream at all. Furthermore, collagen density will be observed in the histopathological image. The results of observations of histopathological preparations of collagen density in the skin tissue of mice in each group can be observed through photos in the following table. The process of reading the collagen density area was observed using the Image J software application. The histopathological images of collagen density in each group are shown in table 3.

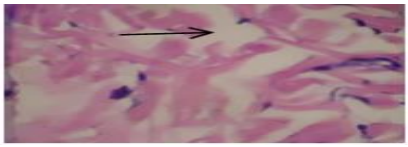
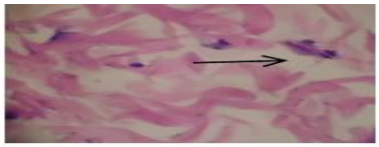
Table 3. Histopathology of Skin Tissue

Group	Histopathology of Collagen and Melanin in Skin Tissue
Control	 1. Collagen density (left) in the control group has a score of 0 (normal) which is purplish blue and looks dense and normal. The condition of collagen density looks denser and the fibers are not scattered because this group was not given any treatment, the mice were only exposed to ultraviolet-B light for 14 days. 2. For the results of damaged skin melanin pigment (right) is seen in the epidermis of the mouse skin in large quantities. This shows that the intensity of exposure obtained also affects the production of melanin pigment that is not given any treatment.
Treatment 1	 1. collagen density in treatment group 1 has a scoring result of 2 moderate increase, the image is purplish blue and looks dense and the fibers look scattered. in this group the mice were exposed to ultraviolet B light and applied

with sambiloto leaf extract cream (*Andrographis paniculata*) with a concentration of 2.5% every day for 14 days.

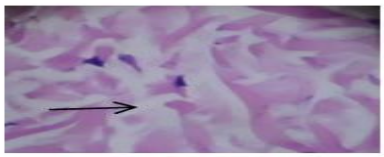
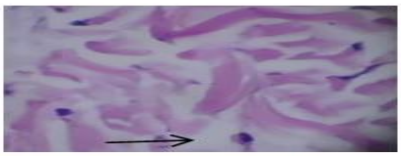
2. For the results of damaged skin melanin pigment (right) seen in the epidermis of the mouse skin, there are fewer grains and it looks like it is starting to fade. This shows that the intensity of exposure also affects the production of melanin pigment which is then also applied with sambiloto leaf extract cream (*Andrographis paniculata*) with a concentration of 2.5% every day for 14 days

Treatment 2

1. Collagen density in treatment group 2 (left) had a scoring result of 3 significant increases (Substantial increase), the image was purplish blue and looked dominant with and collagen density began to look superior compared to treatment group 1. In this group, mice were exposed to ultraviolet B light and applied with Andrographis paniculata leaf extract cream with a concentration of 5% every day for 14 days.
2. For the results of damaged skin melanin pigment (right) seen in the epidermis of mouse skin, there are fewer grains and they are smaller. This shows that the intensity of exposure obtained also affects the production of melanin pigment which is then also applied with sambiloto leaf extract cream (*Andrographis paniculata*) with a concentration of 5% every day for 14 days.

Treatment 3

1. Collagen density in treatment group 3 (left) has a score of 3 significant increases (Substantial increase), the image is purplish blue and looks dominant, the results look like treatment 2. In this group, mice were exposed to ultraviolet B light and applied with Andrographis paniculata leaf extract cream with a concentration of 10% every day for 14 days.
2. For the results of damaged skin melanin pigment (right) visible on the epidermis of the mouse skin, the grains are almost invisible. This shows that the intensity of exposure obtained also affects the production of melanin pigment which is then also applied with sambiloto leaf extract cream (*Andrographis paniculata*) with a concentration of 10% every day for 14 days.

DISCUSSION

Antioxidant activity in the methanol extract of sambiloto leaves (*Andrographis paniculata*) is suspected because based on the phytochemical test it is positive for containing triterpenoid, flavonoid and tannin compounds. This is supported by previous research that triterpenoids are known to have biological activity as antioxidants (Ulfa, 2016). Tannin compounds are a group of polyphenol compounds that have the potential as antioxidants. Free radical products formed in tannin compounds will be stabilized by resonance so that they can function as effective antioxidants (Wijaya, 2011). Phenolic compounds have OH groups that bind to benzene. The OH group is weakly acidic because it has a labile bond (tends to break away), while the DPPH radical is composed of benzene. Benzene has a ring that has resonance, so it is difficult to react/add. This causes polyphenol compounds to easily donate their hydrogen atoms. Triterpenoids, flavonoids and tannins are derivatives of

phenolic compounds, namely compounds that have many hydroxyl groups (OH). This phenolic compound could donate hydrogen atoms so that DPPH free radicals can be reduced to a more stable form. Fitriyani 2009 stated that flavonoids are a group of active compounds that have the potential as natural antioxidants.

The mechanism of flavonoids as antioxidants can be direct or indirect. Flavonoids as antioxidants directly donate hydrogen ions so that they can stabilize reactive free radicals and act as direct radical scavengers (Prawirodiharjo, 2014). Flavonoids as antioxidants indirectly work in the body by increasing the expression of endogenous antioxidant genes through several mechanisms such as increasing the expression of antioxidant genes through the activity of nuclear factor erythritol 2 related factor 2 (Nrf2) so that there is an increase in genes that play a role in the synthesis of endogenous antioxidant enzymes such as SOD (Superoxide dismutase). Triterpenoid compounds, flavonoids and tannins are groups of secondary antioxidants. The mechanism of secondary antioxidants is by cutting off the chain oxidation reaction of free radicals or by capturing free radicals so that free radicals will not react with cellular components. Phenolic compound antioxidants can stop or inhibit the initiation stage by reacting with fatty acid radicals or inhibiting propagation by reacting with peroxy radicals or alkoxy radicals. Therefore, the higher the phenolic compound content in the extract will provide greater peroxide inhibition (Prawirodiharjo, 2014).

CONCLUSION

1. The active substance contained in the extract of sambiloto leaves (Andrographis paniculata) which was tested through phytochemical testing is that the extract of sambiloto leaves (*Andrographis paniculata*) contains secondary metabolites such as alkaloids, flavonoids, saponins, and tannins.
2. The results of the antioxidant test of bitter leaf extract (Andrographis paniculata) through DPPH testing obtained an IC_{50} value of 53.65. So, the antioxidant test of bitter leaf extract (*Andrographis Paniculata*) shows that there is antioxidant content in the bitter leaf extract (Andrographis paniculata) with a strong category.
3. Looking at the antiaging activity of Andrographis paniculata leaf extract with the best collagen density area is group P3 and P2, namely the treatment group given Andrographis paniculata leaf extract cream with a concentration of 5% and 10% because the collagen density is already very large and dense in the histopathology of skin tissue. Followed by treatment group 1 with Andrographis paniculata leaf extract cream 2.5%. Then the average percentage of the worst collagen density area is the control group without any treatment.

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