Antioxidant and Anti-Aging Activity Test Pagoda Flower (*Clerodendrum Paniculatum L*)

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ABSTRACT

This study was conducted to test the antioxidant and anti-aging activity of pagoda flowers (Clerodendrum paniculatum L.). Antioxidant testing of pagoda flower extract through DPPH testing. Seeing the anti-aging activity of pagoda flower extract through differences in collagen density between the Control group without extract and treatment group 1 with 10% pagoda flower cream extract concentration, treatment 2 with 15% concentration, and treatment with 20% concentration of pagoda flower extract cream through DPPH testing obtained an IC50 value of 53.65 indicating strong antioxidant results. For the anti-aging activity of pagoda flower extract, the best collagen density area is group P3 and P2 because the collagen density is already very large and dense in the histopathological picture of skin tissue. The normality test uses Kolmogorov Smirnov with normally distributed data results p> 0.05. Then the homogeneity results using Levene's Test: the p-value is 0.675. So, the conclusion is that there is a significant difference in the average (mean) percentage of collagen density from the four groups.

Keywords : Antioxidant, Antiaging, Pagoda Flower, Collagen

INTRODUCTION

Antioxidants are chemicals that function to fight the adverse effects of free radicals. Free radicals outside the body can come from exhaust fumes, radiation, cigarettes, and toxic substances such as pesticides. In general, free radicals cause the oxidation process in the human body, resulting in cell damage in the human body. The consequences are premature aging and the emergence of degenerative diseases. The main factor of extrinsic skin aging, known as photoaging, is exposure to sun ultraviolet (UV) rays for an extended period. Reactive oxygen species (ROS) factors or excessive exposure to ultraviolet sunlight will activate the elastase enzyme, the only enzyme capable of destroying elastin. Direct

interaction with UVB rays consists of cross-linking adjacent pyrimidine bases, which causes direct damage to DNA and bonds with aromatic amino acids, which cause the formation of free radicals. Anti-aging is any action or strategy to slow aging and maintain optimal health and body function as we age. To counteract the effects of free radicals, humans must consume foods rich in antioxidants every day, even in the human body. Various antioxidants from plants such as chocolate, tea, coffee, and certain herbs can be obtained. Some types of herbal plants that contain antioxidants include pagoda leaf extract (Clerodendrum Paniculatum L) because it is one of the species of the genus Clerodendrum, which is one of the verbenacea tribe that is rich in the diversity of secondary metabolite content that has antioxidant content.

LITERATURE REVIEW

The skin is the organ located on the outermost part of the human body; the protective function of the skin occurs through several biological mechanisms, such as the continuous formation of the horny layer (keratinization and release of dead skin cells) (Djuanda, 2019). Antioxidants are reducing agents that can reduce skin aging by neutralizing reactive oxygen species (ROS) that have been formed. In addition to carotenoids, vitamin C, and vitamin E, some plants can be used as natural antioxidants To counteract the effects of free radicals, humans must consume foods rich in antioxidants every day, even though the human body naturally produces antioxidants (Syaputri, Girsang and Chiuman, 2022). Anti-aging does not aim to stop aging but to extend life span and reduce the risk of heart disease, diabetes, cancer, and other diseases often associated with age. Antioxidant compounds can protect human body cells and prevent oxidative stress caused by free radicals (Nofitasari, L., 2017). Pagoda flower plant (Clerodendrum Paniculatum L). can grow in highland areas with an altitude of 1,300 meters above sea level, and all species of this flower can grow in almost all tropical habitats, tropical rainforests, bushes, and places close to water sources (Princes, 2023). The secondary metabolites of this genus have various biological functions, such as preventing viruses from attacking plants and acting as protectors and insecticides.

METHODS

This study analyzed the antioxidant and antiaging activity of pagoda flower extract (Clerodendrum paniculatum L.) and histopathological features of skin tissue in the antiaging process of white skin of male Wistar strains. This study was conducted at the laboratory of

the pharmaceutical pharmacology department, medicine faculty, and anatomical pathology laboratory at Universitas Sumatera Utara. This study was conducted in May - July 2024. The study sample used male Wistar strain rats (Rattus norvegicus). Researchers used the 3R Principle (Replacement, Reduction, and Refinement), namely Reduction, in determining the number of research samples (Kendall et al., 2018). All research samples, namely 24 male rats, will be divided into 4 groups. The research procedures include acclimation of test animals for 7 days in the laboratory, making pagoda flower extract in cream preparations with concentrations of 10%, 15%, and 20%, testing the phytochemical content of pagoda flower extract, IC50 calculation as a parameter for antioxidants using the DPPH method. The antiaging activity was tested by looking at the collagen density in the skin of mice exposed to light Ultraviolet type B (UVB) for 14 days. Histopathological observations of skin tissue were carried out, and a score was given based on the observation results. Wrinkle scoring was then compared between the skin of mice that had been exposed to UVB rays without pagoda flower extract cream and then compared with treatment groups 1, 2, and 3, which were given pagoda flower cream extract with concentrations of 10%, 15%, and 20%. Then, the mouse skin was taken for electron microscopic observation using histopathological observations of skin tissue to observe the density of skin collagen exposed to UVB rays.Ultra violet type B (UVB).The process of taking skin samples was carried out by biopsy in the back area where the skin would be taken and 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. The data was then tabulated and presented descriptively. The analysis of data normality was used in this study, namely the Kolmogorov-Smirnov test. Data is typically distributed if $p > 0.05$. After conducting the data normality test, a homogeneity test was continued using Levene's test. Data is declared homogeneous if $p > 0.05$ (Ghozali, 2018). Data that has gone through normality and homogeneity tests are then processed again using the t-test to see the comparative differences between groups.

RESULTS

The test animals were divided into 4 groups, the control group was only given base cream, treatment group 1 was given pagoda flower extract cream (Clerodendum Paniculatum) with different concentrations, namely 10%, 15%, and 20%. The active substance contained in the

pagoda flower extract (*Clerodendrum paniculatum L.*) which was tested through phytochemical testing was the pagoda flower extract which contained secondary metabolites such as alkaloids, flavonoids, saponins, and tannins.

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 (Ginting et al., 2020). In the overall test, the IC50 value of pagoda flower extract was 53.65. indicating that the antioxidants in pagoda flower extract are strong with a ppm concentration ranging from 50-100. In making pagoda flower extract cream, the flowers will be used. The process of making pagoda flower extract uses maceration techniques. Dried pagoda flower powder is extracted using 96% ethanol for five days. The ethanol content is evaporated using a rotary evaporator to obtain a thick extract. . The evaporation results are then thickened using a water bath. The resulting thick extract is used to make cream in 10%, 15% and 20% preparations.

In antiaging testingUltraviolet light exposure on the skin determines the anti-aging activity of the cream, if there are more wrinkles it indicates that the cream does not work well on the skin, which means low anti-aging activity. From the results after the mice were exposed to ultraviolet b on the shaved back area of the mice, thenobtained the average percentage of collagen density in the control group of mice (K) without treatment only exposed to ultraviolet b light for 14 days had an average result with a standard deviation of 36.98 ± 1.004, for treatment group 1 (P1) mice exposed to ultraviolet b light and smeared with pagoda flower extract cream with a concentration of 10% every day for 14 days had an average result with a standard deviation of 42.97 ± 1.126, for treatment group 2 (P2) mice exposed to ultraviolet b light and smeared with pagoda flower cream with a concentration of 15% every day for 14 days had an average result with a standard deviation of 51.06 ± 1.465, and for treatment group 3 (P3) mice exposed to ultraviolet b light and smeared with pagoda flower extract cream with a concentration of 20% every day for 14 days had an average result with a standard deviation of 56.96 ± 1.712.

For histopathological observations in the image above, namely in treatment groups 2 and 3, namely mice exposed to ultraviolet b light and smeared with pagoda flower extract cream (*Clerodendrum paniculatum L.)* with a concentration of 15% and 20% every day for 14 days. The condition of collagen density is already very large and dense. While for histopathology, the results of skin melanin pigment showed significant changes between treatment groups,

seen from the skin melanin pigment damaged by ultraviolet b light for 14 days, for the control treatment, the longest healing of skin pigment was seen, while treatment group 3 showed the fastest healing of skin melanin pigment. This shows that the intensity of exposure obtained also affects the production of melanin pigment which is then also smeared with pagoda flower extract cream (*Clerodendrum paniculatum L.*) with a concentration of 20% every day for 14 days.

Reporting Research Results

The hypothesis in this study is that there is a test of the antioxidant and anti-aging activity of pagoda flowers. The conceptual framework of the research can be seen in Figure 1 below.

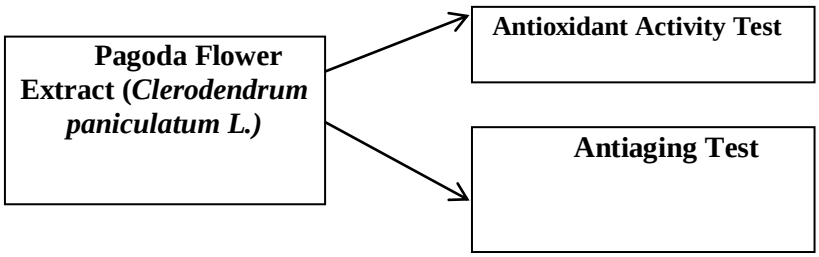


Figure 1. Conceptual Framework

The pagoda flower plant (*Clerodendrum paniculatum* L.) is considered a medicinal plant because of its many properties. The results of the phytochemical test of pagoda flower extract can be seen in the following Table 1.

Table 1. Phytochemical Screening Results of Pagoda Flower Extract

Secondary Metabolites	Color	Results
Flavonoid	Red	+
Saponins	Blue and foamy	+
Tannin	Blackish green	+
Alkaloid	Yellow	+
Triterpenoid	Green	-

From the results of the research above, it is proven that the extract pagoda flower (*Clerodendrum paniculatum* L.) has pharmacological activities such as anti-aging, antioxidant, anticancer and also anti-inflammatory and bacterial activities. Because the

content in it contains alkaloids, flavonoids, saponins, and tannins where the content is good and can be categorized as medicinal plants.

In antioxidant testing, the DPPH method is used because it is easy, the procedure is fast and precise (Ginting et al., 2020). The results of the antioxidant test of pagoda flower extract can be seen in table 2 and the IC_{50} value criteria can be seen in table 3.

Table 2. Pagoda Flower Extract Testing IC_{50}

Sample name	Concentration (ppm)	Percentage Damping	IC_{50} (ppm)
Pagoda Flower	20	28.92	53.65
	40	39.98	
	60	59.97	
	100	85.75	

Table 3. Categories of Concentration Results (Ppm) in Pagoda Flower Extract

Category	Concentration (ppm)
Very strong	< 50
Strong	50-100
Currently	101-150
Weak	151-200
Very weak	>200

So, the table above shows that antioxidants in pagoda flower extract are potent, with ppm concentrations ranging from 50-100. Antioxidants are chemical compounds that can donate one or more electrons to free radicals so that free radicals can be suppressed. In the anti-aging pagoda flower test, 24 mice were used as experimental animals, divided into 4 groups, including the control group, treatments 1, 2, and 3. With the control group not being given

any treatment, treatment group 1 (P1) was given pagoda flower extract (Clerodendrum paniculatum L.) with a concentration of 10%, treatment 2 (P2) 15%, and treatment 3 (P3) with a concentration of 20%. Next, skin observation is carried out by looking at wrinkles, and then the skin is observed through the density of collagenization after post-UVB irradiation. The skin sampling process is carried out by biopsy in the back area where the skin will be taken and cleaned of fur; the skin is 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 are made, and the amount of collagen and melanin is calculated as post-test data. The remaining unused mouse organs will be buried. The results of the collagen density test on mouse skin tissue can be seen in Table 4.

Table 4. Results of Collagen Density Test on Mouse Skin Tissue

Repetition				
	K	**P1**	**P2**	**P3**
1	38.41	42.23	51.68	57.86
2	36.38	41.28	49.89	56.68
3	37.45	42.64	51.64	57.73
4	36.39	43.65	48.99	54.29
5	35.67	44.32	51.02	56.01
6	37.58	43.71	53.15	59.23
Score	**0**	**2**	**3**	**3**
Mean	**36.98**	**42.97**	**51.06**	**56.96**
SD	**1,004**	**1.126**	**1,465**	**1,712**

Scoring information:

0= normal;

1= mild increase

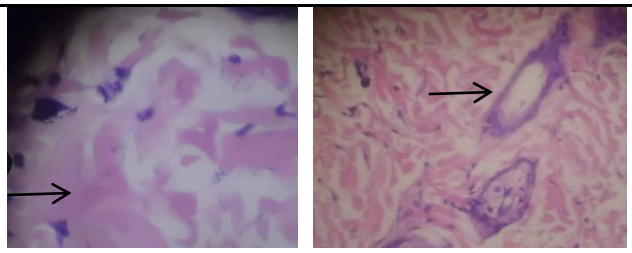
2= moderate increase

3= significant increase (Substantial increase)

The best average percentage of collagen density area was group p3, the treatment group given pagoda flower extract cream (*clerodendrum paniculatum* l.) with a concentration of 20%. The worst average percentage of collagen density area was the control group, namely the group that was only given ultraviolet b light exposure treatment but was not given pagoda flower extract cream (*clerodendrum paniculatum* l.) at all.

The results of histopathological observations of collagen density in the skin tissue of mice in each group can be observed in the photos in the following table 5.

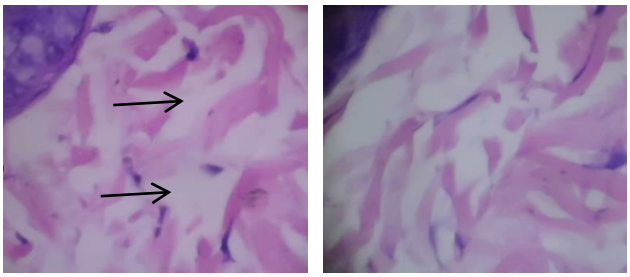
Table 5. Histopathological Description of Skin

Group	Histopathology of Collagen and Melanin in Skin Tissue
Control	

From the results of the histological image by light microscopy with 400x magnification, it can be seen

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.

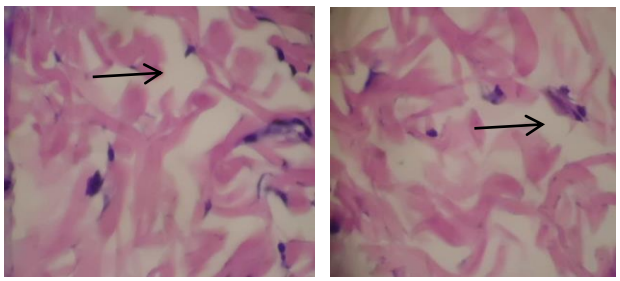
P1



From the results of the histological image by light microscopy with 400x magnification, it can be seen

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 pagoda flower extract cream with a concentration of 10% 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 pagoda flower extract cream with a concentration of 10% every day for 14 days.

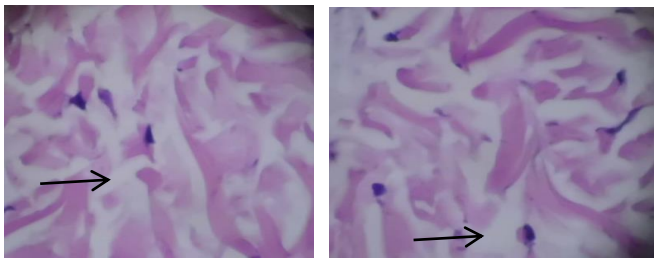
P2



1. Collagen density in treatment group 2 (left) has a scoring result of 3 significant increases (Substantial increase), the image is purplish blue and looks dominant with and collagen density begins to look superior compared to treatment group 1 in this group the mice were exposed to ultraviolet B light and applied pagoda flower extract cream with a concentration of 15% 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 black rice extract cream with a concentration of 15% every day for 14 days.

P3



1. Collagen density in treatment group 3 (left) has a scoring result of 3 significant increases (Substantial increase), the image is purplish blue and looks dominant, the results look similar to treatment 2. In this group, mice were exposed to ultraviolet B light and applied with pagoda flower extract cream with a concentration of 20% 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 barely visible. This shows that the intensity of exposure obtained also affects the production of melanin pigment which is then also applied with pagoda flower extract cream with a concentration of 20% every day for 14 days.

In presenting data analysis, the results of the data normality test in this study can be seen in the following table 6:

Table 6. Kolmogorov-Smirnov test for normality

Group	df	Sig
Control (P0)	6	.200
P1	6	.200
P2	6	.200
P3	6	.200

Based on the results of the normality test that has been carried out using the Kolmogorov-Smirnov Test. a significance result of 0.200 was obtained in all groups that had been

measured in a total of 6 mice for each treatment group. Data is said to be normally distributed if the p value> 0.05. Therefore, it can be concluded that the data is normally distributed.

Then the homogeneity test between groups was carried out using the Levene test with a significance level of 5%. The results of the data homogeneity analysis test data can be seen in Table 7.

Table 7. Homogeneity Test Results

Levene static	**df1**	**df2**	**Sig**
.518	3	20	.675

Based on the data in the table in the "Sig." column, the p-value is 0.675. Thus, at a real level > 0.05, Ho is rejected, so the conclusion obtained is that there is a significant difference in the average (mean) percentage of collagen density of the four groups.

T-test is a statistical method used to test whether there is a significant difference between two groups or populations. With the one sample test model, the following table 8 shows the t-test results below:

Table 8. T-test

t	**df**	**Sig.**	**Mean Difference**
29,182	23	.000	46,995

From the results of the t-test above, it can be seen that the average value is 46.995 from each group and the sig value (2 tailed) is 0.000 < 0.05, so it is concluded that there is a significant (real) difference between each group.

DISCUSSION

Antioxidants are chemicals that function to fight the adverse effects of free radicals (Syaputri, Girsang and Chiuman, 2022). Internal factors of free radicals include skin tissue damage due to thinning skin cells, collagen, and elastin fibers, which are responsible for keeping the skin young. In general, free radicals cause oxidation processes in the human body, resulting in cell damage in the human body (Estevania dan Yusmarlisa, 2018). The consequences are

premature aging and the emergence of degenerative diseases. The main factor of extrinsic skin aging, known as photoaging, is exposure to sun ultraviolet (UV) rays for an extended period (Xu et al., 2022). Sunlight (Ultra Violet) causes clinical and biological skin changes, ranging from immediate side effects such as eye burns, tanning, and hyperpigmentation to long-term side effects such as photoaging and skin cancer. According to several studies, antioxidants can inhibit platelet aggregation and lipid peroxidation and function as antiaging (Nur Zadila, 2021).

Several types of herbal plants contain antioxidants, one of which is pagoda leaf extract (Clerodendrum paniculatum L.).The secondary metabolites of this genus have various biological functions, such as preventing viruses from attacking plants and acting as protectors and insecticides (Yos Banne et al 2023). From the results of phytochemical testing, extracting pagoda flowers (Clerodendrum paniculatum L.) has pharmacological properties such as anti-aging, antioxidant, anticancer, anti-inflammatory, and bacterial properties because the content it contains alkaloids, flavonoids, saponins, and tannins where the content is good and can be categorized as medicinal plants.

After the cream extract preparation is available, testing on mice is carried out to see the collagen density in the group of mice exposed to ultraviolet B light for 14 days. It can be seen that the average percentage of the best collagen density area is group P3, namely the treatment group given pagoda flower extract cream (Clerodendrum paniculatum L.) with a concentration of 20%. Then, the average percentage of the worst collagen density area is the control group, which is only given ultraviolet b light exposure but not pagoda flower extract cream.

Based on the histological description of skin collagen, it can be concluded that the best average percentage of collagen density area is treatment groups 2 and 3, namely mice exposed to ultraviolet b light and smeared with pagoda flower extract cream (Clerodendrum paniculatum L.) with a concentration of 15% and 20% every day for 14 days. The condition of collagen density is already extensive and dense. For the histological results of skin melanin pigment, significant changes were seen between treatment groups, seen from the skin melanin pigment damaged by ultraviolet b light for 14 days; for the control treatment, the longest healing of skin pigment was seen, while the treatment group 3 showed the fastest healing of skin melanin pigment.

This is in line with previous research (Ihsanul Hafiz, 2019). Ethanol extract from pagoda flowers has anti-inflammatory properties at a dose of 100 mg/kg BW given to rat samples, and this extract also has antioxidant content that can repair skin exposed to UVB rays.

CONCLUSION

In this study, it was found that there was antioxidant activity in pagoda flower extract and anti-aging activity which was tested on experimental animals, namely Wistar strain rats exposed to ultraviolet b light by observing skin collagenization and melanin levels as well as histopathological features of rat skin in the experimental group.

The active substance contained in pagoda flower extract (*Clerodendrum paniculatum* L.) which was tested through phytochemical testing is the extract pagoda flower There are secondary metabolite contents such as alkaloids, flavonoids, saponins, and tannins.

The results of the antioxidant test of pagoda flower extract (*Clerodendrum paniculatum* L.) through DPPH testing obtained an IC_{50} value of 53.65. Therefore, the antioxidant test of the extract pagoda flower shows that the antioxidant content in pagoda flower extract is in the strong category.

There is antiaging activity in pagoda flower extract (*Clerodendrum paniculatum* L.) with the best collagen density area being groups P3 and P2 with concentrations of 20% and 15% because the collagen density is already very high and dense in the histopathological picture of skin tissue.

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