Antioxidant And Anti Aging Activity Test Of Cloves (*Syzygium Aromaticum*)

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ABSTRACT

This study aimed to investigate the anti-aging effects of clove extract on collagen density and its antioxidant activity in mice exposed to UVB light..

This study is a True experimental study, with the selection of the type of research design used being Post Test Control Group Design, I used a control group and a treatment group given clove extract after UVB exposure.

The total number of test animal samples in this study was 24. Test animals were grouped randomly into 4 test groups, and 6 animals in each group.

This study concluded that the results of phytochemical analysis showed that clove extract contains flavonoids, saponins, alkaloids, steroids, and tannins, with flavonoids as the key to increasing collagen density. The antioxidant activity of clove extract is intense, with an IC50 value of 17.62 ppm, included in the category of powerful antioxidants. Based on the significance test using One-Way ANOVA, it is known that the control and treatment groups have significant differences in collagen density. The treatment group has a denser collagen density than the control group, and the results of histopathological observations show that the control group produces collagen growth that is included in the thin category. In contrast, the treatment group that was smeared with clove extract is included in the dense category. These results indicate that clove extract, with its high antioxidant capacity, can be a potential anti-aging agent by increasing collagen density.

Keywords: *Antioxidant, Antiaging, Clove Extract*

INTRODUCTION

Having healthy and youthful-looking skin is one of the things that every individual desires. Today, skincare routines have become an integral part of people's lives across age groups. Many individuals realize that skincare is an important investment to keep their skin healthy and free from skin-related diseases and signs of aging ((Ganguly, 2022)). Aging is a complex

process and is a significant risk factor in the development and progression of many disorders. As the world's population ages, age-related chronic diseases will become more common and majorly impact quality of life. The pathogenesis of many diseases, including cardiometabolic disorders, neurodegenerative diseases, and cancer, consists of the accumulation of reactive oxygen species that cause oxidative stress and inflammation, the main causes of cellular aging (Rusu, 2022)). Sunlight exposure is essential for vitamin D synthesis, but exposure to harmful UV rays causes premature aging, forming reactive oxygen species (ROS). Reactive oxygen species (ROS) are molecules capable of living independently, containing at least one oxygen atom and one or more unpaired electrons. This group includes oxygen-free radicals. Under physiological conditions, small amounts of ROS are formed during cellular processes, such as aerobic respiration or inflammatory processes, especially in hepatocytes and macrophages. ROS are signaling molecules. In addition, they induce cell differentiation and apoptosis, thus contributing to the natural aging process (Hofmann E, 2023)). There are many defense mechanisms to prevent the formation of reactive oxygen species (ROS) and the damage they cause. These mechanisms are known as “antioxidant defense systems” or simply “antioxidants.” Antioxidants are chemical compounds that donate electrons to unpaired free radicals, thereby reducing the oxidative effects of natural exogenous free radicals. They have been clinically proven to be effective as antioxidants (Valgimigli, 2018). One of these chemical compounds is a phenolic compound, a secondary metabolite that protects plant organs from oxidation. Therefore, phenolic compounds are referred to as natural antioxidants. In addition to their activity as antioxidants, phenolic compounds in plants are known to have anti-carcinogenic, anti-microbial, anti-allergic, anti-mutagenic, and anti-inflammatory properties (Cirmi S, 2017). Another phytochemical that has antioxidant activity is flavonoids. Flavonoids are polyphenolic compounds found in various types of plants and are helpful in maintaining human health. Flavonoids in fruits and vegetables that are regularly consumed can reduce the risk of cardiovascular disease (Ivey KL, 2017). Plants are the primary source of natural antioxidants consumed or used as medicine. Antioxidants are obtained from vegetables, mushrooms, fruits, herbs, cereals, flowers and spices. In addition, antioxidants can also be obtained from businesses that produce agricultural by-products. Flavonoids, lignans, stilbenes, anthocyanins,, several other polyphenolic compounds, vitamins,, and carotenoids such as carotene and xanthophylls are obtained and derived from plants. Natural antioxidants have many pharmacological properties such as anti-cancer, anti-viral, anti-inflammatory, and anti-bacterial (Alamzeb, 2024)). One of the plants that has antioxidant content is cloves. Cloves have been used for centuries in both traditional medicine and cooking, and their essential oils have been used in perfumery, folk remedies, and food flavorings. Cloves have traditionally been associated with boosting the immune system and increasing disease

resistance. Cloves are also still used to treat various health problems as an anesthetic, antiseptic, antiviral, antifungal, and antimicrobial. In addition, clove essential oil has been used to treat burns, gum infections, digestion, breathing, and other diseases. Previous studies have shown additional essential characteristics such as inflammatory, antimutagenic, and antioxidant capabilities. Other studies using crude extracts of clove shoots and leaves have also shown potential as antioxidant agents (Lesmana, 2021). Based on this background, researchers are interested in testing clove extracts' antioxidant and antiaging activities in white Wistar rats exposed to UVB light.

LITERATURE REVIEW

Human skin is composed of various tissues that work together as a structure to maintain the body's internal conditions (homeostasis) and function together as a communicator and defense against the outside world. The skin is a dynamic and constantly changing organ involved in many processes important to our health, such as body temperature regulation, fluid balance, sensory reception, and vitamin and hormone synthesis. Human skin comprises three distinct compartments, the epidermis, dermis, and hypodermis (E, 2023) Ultraviolet radiation is emitted naturally from the sun and artificial sources and can cause severe damage to the skin, known as sunburn. The primary sources of artificial ultraviolet radiation are mercury vapor lamps, water-cooled lamps, and air-cooled lamps, which are mainly used for diagnostic and therapeutic purposes. Chronic exposure to ultraviolet radiation causes skin browning and accelerates skin aging, which is called photoaging. Ultraviolet radiation from sunlight can be classified into three types based on their wavelengths: UVA (320–400 nm), UVB (280–320 nm), and UVC (100–280 nm) (Lephart, 2018)). Among them, almost all UVC and some UVB are absorbed by the ozone layer and not affect human skin (O'Connor et al., 2021). The remaining UVB can penetrate the skin's epidermis and cause erythema (sunburn), while UVA can attack the dermis and is responsible for about 98% of severe skin aging (Mesa-Arango, 2017). UVB rays absorbed by epidermal cells cause DNA damage, increase oxidative stress and reactive oxygen species (ROS), and cause premature aging. In contrast, UVA has a higher wavelength that can cause indirect DNA damage and degradation of collagen and elastin fibers through oxidative stress pathways. Overall, chronic exposure to UVR causes an increase in nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and produces ROS, which increases inflammation, cytokines, chemokines, and skin aging. Chronic and persistent inflammation caused by UVR can weaken the skin's defense mechanisms degrade collagen and elastin fibers, and ultimately cause premature aging. This review aims to discuss the inflammatory molecules associated with UVR-mediated skin aging. In this review, our primary focus is to outline the effects of UVR on epidermal keratinocytes and dermal

fibroblasts in the skin aging process (Ansary TM, 2021). Antioxidants are a group of small organic molecules and large, structurally diverse enzymes consisting of complex systems with overlapping activities that work synergistically to enhance cellular defenses and counteract oxidative stress caused by various reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Moussa, 2019) Natural antioxidants are very good for skin health, these antioxidants come from plants, one of which is the clove plant, Cloves (*Syzygium aromaticum*) are one source of natural ingredients that have various biological activities that provide benefits for human health. Traditional communities widely use cloves from generation to generation as wound medicine, massage oil, body warmer, and cooking spices. Clove extract has several promising bioactive compounds, such as flavonoids, saponins, phenolics, tannins, steroids, terpenoids, and alkaloids. Clove extract has also been studied to have antibacterial and antifungal activities (Hu, 2018), anti-inflammatory (Nikoui, 2017), analgesic (Razafimamonjison, 2014), antioxidant (Fauzya, 2019.), antiglycation, and anti-aging (Lesmana DA, 2021)

METHODS

This study is a True experimental study, with the selection of the type of research design used Test Only Control Group Design, which is a type of research that only observes the control and treatment groups after being given an action. This study was conducted in June-August 2024. Research stages such as sample preparation, extract preparation, and laboratory tests were carried out at the Laboratory of the Department of Pharmacology and Therapeutics, Faculty of Medicine, University of North Sumatra. In contrast, histopathological analysis was carried out at the Anatomical Pathology Laboratory, University of North Sumatra. Ethical Clearance will be submitted to the Health Research Ethics Commission (KPEK) of Prima Indonesia University and is approved (Approval No. [050/(KPEK-FKKGIK/2024). All procedures comply with animal welfare guidelines to address ethical issues related to animal handling and care. The research sample in this study was male white rats (Rattus norvegicus) of the Wistar strain weighing 160-200 grams and aged 2-3 months. The researcher used 6 Wistar rats for each experimental group so that the total number of test animals in this study was 24. The grouping of test animals was carried out randomly into 4 test groups. The variables used for testing because they were research subjects and were seen when conducting the study, namely the independent variable of clove extract cream and the dependent variable of antioxidant and antiaging activity. The preparation of clove extract refers to previous studies that have been conducted. The preparation of clove extract was modified by Taher et al. (2018) by grinding clove flowers and stems to a size of 60-70 mesh, followed by triple ethanol extraction. The resulting filtrate was concentrated using a rotary evaporator to obtain

a paste, which was used to formulate clove extract cream with concentrations of 0%, 2.5%, 5%, and 10%. The ingredients were weighed according to the table and added with distilled water until the total weight was 100 grams.

Table 1 Clove Leaf Extract Cream Preparation Formula (O/W)

Material Name	Cream Formula (grams)			
	F0	F1	F2	F3
Clove Extract	**0**	**2.5**	**5**	**10**
Cetyl alcohol	4	4	4	4
Glycerin	15	15	15	15
TEA (triethanolamine)	3	3	3	3
Stearic acid	12	12	12	12
Methyl paraben	0.2	0.2	0.2	0.2
Propyl paraben	0.02	0.02	0.02	0.02
Aquades until.	100	100	100	100

Antioxidant Activity Test

There are many bioanalytical methods for measuring antioxidant effects, including 1,1-diphenyl-2-picrylhydrazyl (DPPH). DPPH is the most popular method and is commonly used to determine antioxidant capacity (Gulcin et al., 2023). Antioxidant activity was identified using the DPPH method. The inhibitory ability of the extract against DPPH was measured using a UV-Vis spectrophotometer at a wavelength of 515 nm. The results of the DPPH trapping method are calculated by calculating IC50. This value indicates that the plant extract can cause a 50% reduction in DPPH activity. This can also be seen from the color change of the test sample, which is dark purple when DPPH is added; it will change to yellowish if the extract has a reduction. The calculation results are entered into the regression equation with the sample concentration (ppm) as the abscissa (X-axis), and the percentage value of the reduction activity as the ordinate (Y axis). The parameters of skin aging in this study were seen by looking at the increase in the amount of collagen in the skin of mice.Histopathological scoring for collagen tissue distribution density was performed based on the calculation of 1 field of view, at 400x magnification.

0= No collagen fibers found

+1= Low collagen fiber density (less than 10%)

+2= Medium collagen fiber density (10-50%)

+3= Dense collagen fiber density (50-90%)

+4= Collagen fiber density is very dense (90-100%)

The antioxidant observation data were tabulated and determined based on their inhibitory activity. The data in this study were obtained from the results of absorbance measurements of clove extract using a spectrophotometer. The antiaging observation data in the form of collagen tissue density through microscopic examination were collected and then scored. The research data were tabulated, and then the changes found were analyzed and presented descriptively.

RESULTS

Results Overview of Test Animals

The characteristics of the test animals used in the research are as follows:

Table 2 Characteristics of test animals

Component	Group			
	Control	P1	P2	P3
Types of Rats	*Rattus norvegicus*white wistar strain			
Gender	Male			
General Condition	Healthy and active			
Average Initial Body Weight	191gr	192gr	188gr	183gr
Average Final Weight	190gr	190gr	187gr	182gr

Based on the characteristics of the test animals, in general, the mice were in a healthy condition during this study, namely before and after the treatment. The average initial body weight of the mice before treatment was 191 grams in the control group, 192 grams in treatment group 1, 188 grams in treatment group 2, and 183 grams in treatment group 3. After the treatment ended, the mice were weighed again, and the mice only experienced a slight decrease in body weight, which means that the mice remained in a healthy condition during the study. Then the researchers began the treatment process. The treatment process began by shaving the fur on the back of the mice. First, the mice were made unconscious using 50 mg/kgBW ketamine so that the mice did not move during the shaving process. After losing consciousness, the fur around the back area was shaved clean (bald) according to the desired area (2×2cm). The mice were then exposed to UVB light. Exposure to UVB rays for 14 days and application of clove extract cream was carried out once every 2 (two) days, namely 20 minutes before exposure to

UVB rays (to provide time for topical ingredients to be absorbed into the skin) and 4 hours after exposure to UVB rays (ROS formation begins 4 hours after exposure).

Specific Objective Results

Based on the results of the phytochemical tests conducted, it can be concluded that clove extract (*Syzygium aromaticum*) contains secondary metabolites in flavonoids, saponins, tannins, alkaloids, and steroids. The following are the screening results obtained:

Table 3 Phytochemical Tests

Secondary Metabolites	Color	Results
Flavonoid	Yellow	+
Saponins	Yellow and foamy	+
Tannin	Blackish blue	+
Alkaloid	Yellow	+
Steroid	Green	+

The antioxidant activity of clove extract (Syzygium aromaticum) was tested using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. The DPPH method was chosen because it requires a small sample and is simple, easy, fast, and sensitive to evaluate the antioxidant activity of natural compounds. The antioxidant activity test was carried out using a UV-Vis spectrophotometer. This test was carried out to determine the remaining DPPH absorbance after the extract was added. If a compound has antioxidant activity, there will be a decrease in the DPPH absorbance value at a wavelength of 515.4 nm. The decrease in DPPH absorbance was measured against the control absorbance, namely the DPPH absorbance in methanol pa without adding test material. The decrease in DPPH absorbance is indicated by the degradation of the DPPH color from purple to yellow (Amanda et al., 2019). The DPPH color degradation process is directly proportional to the concentration of the added extract. From the DPPH absorbance value obtained, the percentage value of DPPH radical inhibition (% inhibition) can be determined. From the % inhibition value, the IC50 (inhibitory concentration) value can be determined.

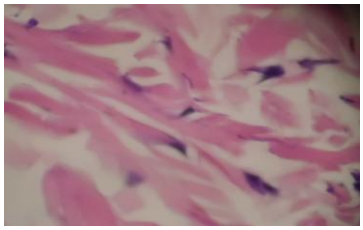
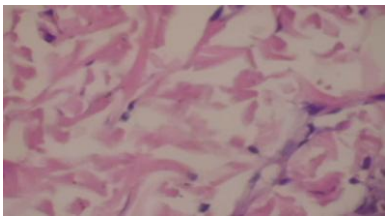
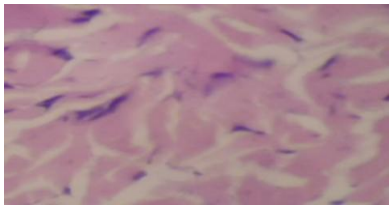
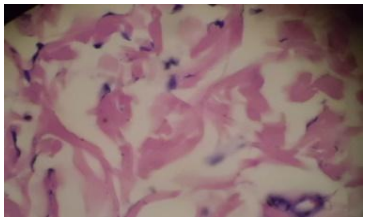
Table 4 Antioxidant test of clove extract

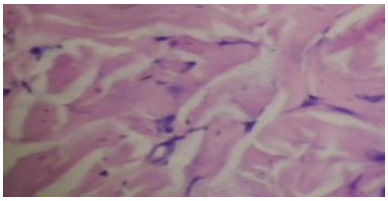
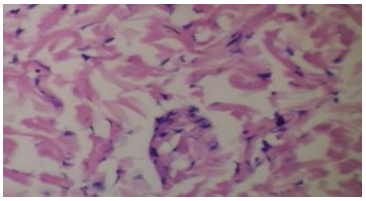
No.	Concentration	Absorbance			Average
		Repetition 1	Repetition 2	Repetition 3	
1.	0		1,305Acontrol)		1,305 (Acontrol)
2	5	0.874	0.852	0.884	

.					0.870
3.	10	0.654	0.689	0.632	0.658
4.	20	0.495	0.462	0.481	0.479
5.	40	0.258	0.242	0.251	0.250

The data from the research results of the clove extract antioxidant activity test is 17.62ppm. The IC50 value is inversely proportional to the strength or antioxidant potential of a material, the lower the IC50 value, the stronger the antioxidant potential. So the antioxidant activity of clove extract is included in the antioxidant category and is very strong.Histopathological assessment for collagen fiber density was performed at 400x magnification.. The purpose of this observation is to see the structure and morphology of cells, especially collagen cells in each skin specimen exposed to UVB light in the control and treatment groups. The results of histopathological examination using a magnification light microscope on skin tissue specimens showed differences in collagen density as follows:

Table 5 Histopathological Description of Skin Tissue

No	Group	Histopathological Image of Skin Tissue	
1	Control (Base Cream)		
2	Treatment 1 (Clove extract cream 2.5%)		
3	Treatment 2 (Clove extract cream 5%)		

4	Treatment 3 (Clove extract cream 10%)		

The results of this study indicate that administration of clove extract cream can affect the density of skin collagen in rats exposed to UVB rays in male white rats (Rattus norvegicus) of the Wistar strain. This is evidenced by the difference in collagen density between the control group and the treatment group. The microscopic appearance of the histopathology tissue of the treated skin is presented in Table. Collagen density based on the calculation of one field of view at 400x microscope magnification is arranged based on scoring; 1 = low collagen fiber density (less than 10% in one field of view, 2 = moderate (10% - 50%), 3 = dense (50% - 90%), 4 = very dense (90% - 100%). The results of the collagen density test using image J software on each group of experimental mice are presented in the following table.

Table 6 Collagen Density Test Results (%)

Repetition	**Group**			
	Control	**Treatment 1**	**Treatment 2**	**Treatment 3**
1	9.21	33.13	66.12	78.12
2	9.34	44.12	65.87	77.55
3	8.93	39.45	70.04	80.01
4	8.12	38.22	73.27	74.22
5	9.43	46.13	68.04	75.12
6	8.25	31.82	67.25	88.31
Mean	**7.34**	**32.57**	**68.43**	**78.88**
Score	**1**	**2**	**3**	**3**

The results showed that the control group that was only smeared with base cream produced a score of 1, which is less collagen fiber density. Treatment group 1 produced a score of 2, which is moderate collagen density. Treatment groups 2 and 3 produced a score of 3, which means that collagen density is in the dense category. The density of collagen formed in the histopathology of the skin of white rats (Rattus norvegicus) of the Wistar strain exposed to UVB light is inseparable from the antioxidant and antiaging activity of clove extract.

DISCUSSION

The purpose of this study was to test and analyze the antioxidant and antiaging activity of clove extract (*Syzygium aromaticum*). The parameter used to see the antioxidant activity is the IC50 value obtained through the DPPH test. Antiaging activity can be seen through histopathological observations in the form of collagen density in the skin of mice exposed to UVB light. There are many compounds from herbs that can be used as natural exogenous antioxidants and have been clinically proven to be effective as antioxidants (Amorati & Valgimigli, 2018). Phenolic compounds are referred to as natural antioxidants. In addition to their activity as antioxidants, phenolic compounds in plants are known to have anti-carcinogenic, anti-microbial, anti-allergic, anti-mutagenic, and anti-inflammatory properties (Cirmi S, 2017). Other phytochemicals that have antioxidant activity are flavonoids. Flavonoids are polyphenolic compounds found in various types of plants and are useful in maintaining human health. Flavonoids, lignans, stilbenes, anthocyanins and several other polyphenolic compounds, vitamins and carotenoids such as carotene and xanthophylls are obtained and derived from plants. Natural antioxidants have many pharmacological properties such as anti-cancer, anti-viral, anti-inflammatory and anti-bacterial (Alamzeb, 2024), One of the plants that has antioxidant content is cloves. Researchers conducted a trial on male white rats (Rattus norvegicus) Wistar strain to prove this hypothesis. The sample in this study was a male white rat (Rattus norvegicus) of the Wistar strain weighing 160-200 grams and aged 2-3 months. Determination of the number of samplesusing the ferderer formula for 4 groups. The number of samples in this study was 24 rats which were divided into 4 groups, randomly the control group which was only smeared with base cream and the treatment group which was smeared with clove extract cream with concentrations of 2.5%, 5%, and 10%. The study was conducted by collecting data related to observations of treatment procedures. First, conduct a phytochemical test. The results of phytochemical tests on clove extract showed that clove extract (Syzygium aromaticum) contains secondary metabolites in the form of flavonoids, saponins, tannins, alkaloids, and steroids. The results of this test are in accordance with previous research conducted by (Dahiru, 2021). The study showed that clove extract contains secondary metabolite compounds in the form of flavonoids, saponins, tannins, alkaloids, and steroids. The results of the test of the antioxidant activity of clove extract were 17.62ppm. This IC50 value is inversely proportional to the strength or antioxidant potential of a material, the lower the IC50 value, the stronger the antioxidant potential. So that the antioxidant activity of cloves is included in the antioxidant category and is very strong. The results of this test are in line with research conducted by (Alfikri FN, 2020) which states that clove extract has antioxidant activity that is included in the very strong category. The researchers then continued the study with an antiaging activity test. UVB exposure 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). Sunlight exposure is essential for vitamin D synthesis, but exposure to harmful UVB rays causes premature aging, the beginning of the formation of reactive oxygen species (ROS). Reactive oxygen species (ROS) are molecules that can live independently, contain at least one oxygen atom and one or more unpaired electrons. This group includes oxygen free radicals. ROS induce cell differentiation and apoptosis, thus contributing to the natural aging process. This research procedure was conducted for 14 days and produced data that needed to be processed and tested first, so it was necessary to do some data analysis in the form of normality, homogeneity, and significance tests. The normality test data was obtained with the help of SPSS using the Kolmogorov-smirnov test. The results showed that the data for each group was normally distributed with a significance value of 0.200 in all test groups for testing urea and creatinine levels. So it can be concluded that the data is normally distributed, or can represent the population. After that, a homogeneity test was carried out to see the subject variance. The results showed that the control and treatment groups came from populations that had the same variance, or both groups were homogeneous with a significance value of 0.057. Finally, the One-Way ANOVA test was conducted to see the significance value. The results of the One-Way ANOVA test in the table above show that the significance value produced is 0.000 or <0.05. Based on these data, it can be concluded that there is a significant difference between the control group and the treatment group. The Post Hoc LSD test is used to determine whether the group has a significant difference from other groups. The results of the Post Hoc LSD test analysis in this study showed a significance value of 0.000 or less than 0.05, which means that the group has a significant difference from other groups. The difference in collagen density in the skin of mice exposed to UVB rays cannot be separated from the content of secondary metabolites in clove extract in the form of flavonoids, saponins, alkaloids, steroids, and tannins which act as free radical transporters that arise due to UVB exposure. The compound that plays a role in increasing collagen density in mice exposed to UVB rays is flavonoids. Flavonoids can act as anti-aging agents that can ward off free radicals that cause wrinkles and other signs of aging. Research shows that flavonoids have good anti-aging activity. Flavonoid compounds have been observed to eliminate senescent cells in vitro, improve physical function, and increase the lifespan of mice in vivo (Xu et al., 2018).

CONCLUSION

1. Phytochemical test results show that eucalyptus extract contains secondary metabolites in the form of flavonoids, saponins, alkaloids, steroids and tannins. These compounds, especially flavonoids, help increase collagen density and act as anti-aging agents in mice exposed to UVB light.
2. The results of the antioxidant activity test showed that clove extract had an IC50 value of 17.62ppm. This value is included in the category of very strong antioxidants.
3. Based on the significance test using One-Way ANOVA, it is known that the control group and the treatment group have significant differences in collagen density. The treatment group has a denser collagen density than the control group.
4. The results of histopathological observations showed that the control group produced collagen growth that was included in the thin category, while in the treatment group that was smeared with clove extract, it was included in the dense category.

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