

THE POTENTIAL OF SUPEROXIDE DISMUTASE ENZYME FROM TOMATO FRUIT (*SOLANUM LYCOPERSICUM*) TO REPAIR COLLAGEN DAMAGE IN 3T3 FIBROBLAST CELLS EXPOSED TO ULTRAVIOLET A RADIATION

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ABSTRACT

UV A radiation can cause photoaging to the skin which is show by a decrease in the amount of collagen and death of fibroblast cell. This study aimed to determine the SOD enzyme activity of tomatoes (*Solanum lycopersicum* Mill.) influences the increase of viability of fibroblast cell and deposition of collagen. The study design used a completely randomized design (CRD) with 11 treatments and 3 repetitions. The procedures of this study include: (1) extracting SOD enzymes (2) making bradford reagents (3) making standard protein solutions (4) measuring total protein content (5) testing the antioxidant activity of SOD enzymes (6) preparing 3T3 fibroblast cells (7) Cell exposure 3T3 fibroblasts with UV A rays (8) preparation of tomato SOD enzyme stock solutions (9) determination of cell viability and collagen deposition. Data analyzed by normality test, homogeneity test, one way ANOVA test, tuckey test and dunnet test. The results of SOD enzyme activity showed the highest percent inhibition was 77.83%. Highest value of percent viability of fibroblast cells that were exposed to UV A rays after given 90% SOD enzyme treatment was 233.00% and collagen deposition was 157.67%. The results showed that variations in the SOD enzyme concentration of tomatoes had a significant effect on cell viability and collagen deposition in fibroblast cells. with a value of sig = 0,000 ($p < \alpha$). Based on the result, we can concluded that SOD enzyme from tomatoe fruit show significant activity as anti-photoaging.

KEYWORDS SOD Enzyme Extract, Fibroblast Cells, Cell Viability, Collagen Deposition, Photoaging, UV A rays



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INTRODUCTION

One of the benefits of UV light is to synthesize vitamin D and kill bacteria. In addition to having benefits, UV light can also cause dermatoheliosis or photoaging if exposed to the skin for too long (Isfardiyana, 2014; Khan et al., 2018). Photoaging due to the effects of UV-A light is referred to as extrinsic skin aging. Photoaging is characterized by skin that appears thicker, drier, uneven surface, hyperpigmentation or hypopigmentation, wrinkles, telangiectasia, premalignant lesions and saggy skin.

UV radiation that causes photo aging consists of UV-A (320 - 400 nm), UV-B (280 - 320 nm) and UV-C (100-280). UV-B has a short wavelength and is mainly absorbed into the epidermis, whereas UV-A reaches the dermis and damages fibroblasts due to its longer wavelength. If the intensity of UV-A rays reaching the earth is 95% while UV-B rays are 5%. UV-A light is 1,000 times more effective at penetrating the skin layer than UV-B light (Indrayati et al., 2016). The concentration of UV-A light has a major influence on the production of *reactive oxygen species* (ROS) free radicals. UV-A light will be absorbed by intracellular chromophores such as riboflavin, porphyrin, nicotinamide, and enzymes located on the cell membrane. Absorption of UV-A light will cause damage to cell tissues and membranes. The damage occurs because the ROS that arise affect fibroblasts by increasing cytokines and reducing the production of transcription factors so that collagen production decreases. Damage also occurs when the enzymes MMP-1 (Matrixmetalproteinase) and elastase are activated by ROS so that collagen and elastin degradation increases.

UVB and UVA induce the formation of reactive oxygen species (ROS) in skin tissues (Kong et al., 2018). High concentrations of intracellular ROS (superoxide, hydrogen peroxide, hydroxyl radical, alkylperoxyl radical) directly attack cell membranes and induce lipid peroxidation. Lipid peroxidation is a process in which ROS degrade polyunsaturated fatty acids from cell membranes. Lipid peroxidation is an important pathophysiological event in photoaging (Khan et al., 2018). UV radiation, especially UV-A rays that cause skin fat peroxidation with its higher wavelength than UV-B, is able to penetrate the *stratum corneum* to the dermis so that a drug delivery system is needed that can carry flavanoid compounds through to the dermis. This can be done by tomato SOD enzyme crude extract.

Naturally, cells secrete the enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) to neutralize ROS. Superoxide dismutase (SOD) is an enzymatic antioxidant produced by eukaryotic and prokaryotic cells. SOD has a role as the main defense in inhibiting superoxide anions. SOD levels in the human body will decrease with age. A person whose natural SOD levels decrease, needs external SOD to help the body ward off free radicals.

External SOD can be obtained from natural materials that contain antioxidant compounds such as tomatoes (*Solanum lycopersicum*). Tomato fruit in addition to being a vegetable is also widely used by the community to treat several diseases

including as anti-inflammatory, anti-cancer, especially prostate cancer, hypertension and coronary heart disease, lowering high cholesterol and LDL levels and improving the quality of spermatozoa.

SOD enzyme activity in tomato fruit that has been studied in several studies shows anticonvulsant activity, and anti-apoptotic neuroprotective. Tomatoes contain vitamin C, Vitamin E, provitamin A carotene, zinc, iron, calcium, and phenolic components flavonoids and phenolic acids. Tomato fruit is the main source of lycopene. Lycopene in tomato fruit acts as one of the most potential antioxidants in stopping oxidative damage caused by UV-A radiation. The action of lycopene as an antioxidant is influenced by concentration, bioavailability, and interaction with other antioxidants.

Based on the above description, this study aims to investigate the potential of superoxide dismutase (SOD) enzyme activity from tomato fruit (*Solanum lycopersicum*) as an antioxidant to counteract free radicals due to UV-A radiation exposure. The research titled "Potential Superoxide Dismutase Activity from Tomato Fruit (*Solanum lycopersicum*) to Repair Collagen Damage in Fibroblast Cells Exposed to Ultraviolet-A Light" focused on exploring the effect of varying concentrations of SOD crude extract on viability and collagen deposition in fibroblast cells exposed to UV-A light, as an effort to improve the effects of skin aging. This research is also a new contribution to the field of free radical scavenging and natural antioxidants, with potential applications in natural ingredient technology for skin anti-aging development.

RESEARCH METHOD

This study used the population of tomato fruit (*Solanum lycopersicum*) from Legi market Surakarta as the main subject. This tomato fruit is determined as a population with the following specifications: Kingdom Plantae, Division Spermatophyta, Sub division Angiospermae, Class Dicotyledonae, Order Tubiflorae, Family Solanaceae, Genus *Lycopersicum*, and Species *Solanum lycopersicum*. The samples used were fresh tomatoes from Legi market, Surakarta. The research method involved the use of independent variables in the form of variations in the concentration of SOD enzyme from tomato fruit, with dependent variables in the form of cell viability and collagen deposition in 3T3 fibroblast cells exposed to UV-A light. The experimental methodology included the extraction of SOD enzyme from tomato fruit, determination of protein content using the Bradford method, testing the antioxidant activity of SOD enzyme, and testing the anti-photoaging activity on 3T3 fibroblast cells in vitro. Materials and tools used included various chemicals, culture media, and laboratory equipment such as a spectrophotometer and CO2 incubator. The results were analyzed by preliminary test, ANOVA test, and post hoc test to evaluate the effect of various concentrations of SOD enzyme on cell viability and collagen deposition.

RESULT AND DISCUSSION

Research Results

Plant Determination

Plant identification was carried out at the Center for Research and Development of Medicinal Plants and Traditional Medicines (B2P2TOOT) Tawangmangu. The identification results can be confirmed that the plants used in this study are *Solanum lycopersicum*. The identification results are attached in appendix 1. Plant identification is the first step that must be done in a study to ensure the correct identity of the plants used because tomato plants have many species, so as to avoid errors caused by sampling (Darliana, 2009).

Tomato samples have morphology in the form of pinnate compound leaves, alternate location, ovate to elongated shape, pointed tip, rounded base, large leaf blade notched edges, smaller leaf blade jagged edges, 10-40 cm long, light green color. Tomato samples are fleshy fruits, the skin is smooth thin shiny, diverse in shape and size, yellow or red in color. Tomato fruits are round, oval, flat round, or oval. Young fruits are light to dark green in color, while old fruits are bright red or dark bright yellowish. Or blackish red, in addition to these colors, there is also a light yellow fruit color. Tomato seeds are flat, hairy, and covered with pulp. The color of the seeds is white, yellowish white, some are brownish.

Extraction of Tomato Fruit (Solanum lycopersicum) SOD Enzyme

SOD enzyme was obtained by extracting tomato fruit. The first SOD enzyme extraction process is done by washing the sample using running water and then soaking it using NaClO for 10 minutes, this aims to remove impurities contained in the sample. Tomato fruit samples were then washed again using aquabides and soaked overnight. The tomato fruit samples were then pulverized using a blender with potassium phosphate buffer as the solvent for approximately 3 minutes. The purpose of pulverization is to expand the surface of the sample which will facilitate the extraction process because the contact between the sample and the solvent will be wider.

Samples that have been mixed well with potassium phosphate buffer pH 7.0 are filtered to separate the pulp and tomato sample solution. The homogenization result of the sample and buffer is a cloudy solution which is then separated between the protein and the residue by centrifugation. The first centrifugation at 3000 rpm for 30 minutes can separate the protein from the mixture. The main principle of centrifugation is to separate substances based on molecular specific gravity by applying centrifugal force so that heavier substances will be at the bottom, while lighter substances will be located at the top.

The addition of ammonium sulfate was varied at concentrations of 20, 40, 60 and 90% in the resulting supernatant. The addition of 20 to 90% ammonium sulfate is better known as the *salting out* method. *Salting out* is a method used to separate proteins based on the principle that proteins are less soluble when in areas of high salt concentration. A high salt concentration is needed to accelerate the release of different solutions from one protein to another. The effect of salt addition on protein solubility varies depending on its concentration and the amount of ion charge in the

solution. The higher the concentration and the number of charge ions, the more effective the salt is in precipitating the protein.

The second stage centrifugation was continued at 3000 rpm for 30 minutes at 4°C, then the supernatant was taken and ammonium sulfate was added to the supernatant with 20% saturation and stirred for 60 minutes using a *magnetic stirrer* in a refrigerator. The solution was then centrifuged again at 6000 rpm for 10 minutes at 4°C and the supernatant and pellet were separated. The supernatant obtained was brought back to 40, 60 and 90% saturation by adding ammonium sulfate and stirring for 60 minutes using a *magnetic stirrer* in a refrigerator. The supernatant was centrifuged at 6000 rpm for 10 minutes and the supernatant and pellet were separated. The extracted tomato was suspended in 0.05 M phosphate buffer pH 7.0 and dialyzed overnight in 4 L of 0.05 M potassium phosphate buffer pH 7.0. The concentrations of ammonium sulfate obtained were concentrations of 20, 40, 60 and 90%.

Determination of Protein Content Using the Bradford Method

The condition of 3T3 fibroblast cells is normal, which is fusiform with branched cytoplasm and cell ends that resemble fibers. Exposure of 3T3 fibroblast cells to Ultraviolet A (UV A) light aims to create damaged cell samples with decreased collagen deposition, which is indicative of photoaging due to UV exposure. This photoaging is influenced by factors such as the level of UV exposure, the amount of melanin, and the geographical conditions in which individuals live (Kammeyer & Luiten, 2015). The results of UV A exposure showed that fibroblast cells had decreased viability and collagen deposition, due to cell damage induced by free radicals from UV A light. This study highlighted that superoxide dismutase (SOD) enzyme from tomato extract, at a certain concentration such as 100 mg/ml, was able to significantly restore fibroblast cell viability, showing a positive impact on collagen deposition. This treatment illustrates that SOD from tomato extract can be potentially an effective antioxidant agent in reducing the adverse effects of photoaging on the skin (Hamid et al., 2010; Pandel et al., 2013).

The Bradford method is used in the determination of soluble protein content in tomato fruit extract. This method is based on the reaction of CBBG dye that forms a complex with protein, producing a blue color that can be measured at a wavelength of 595 nm. The measurement results show that the concentration of soluble protein in various concentrations of tomato extract can be quantified by using the BSA standard curve as a reference. The use of the Bradford method provides fast and accurate results, although it is susceptible to the influence of nonprotein substances such as detergents. The results also showed that the concentration of soluble protein in tomato extracts ranged from 0.008 mg/ml to 0.38 mg/ml, depending on the extract concentration used. These findings provide important insights in understanding the antioxidant potential of tomato extracts in the treatment of skin photoaging through the development of more effective cosmetic or nutraceutical formulations.

Antioxidant Activity Testing of SOD Enzyme Extract

The results of the antioxidant activity test of extra crude SOD enzyme can be seen in the following table:

Table 1. Absorbance of SOD Activity Test Results Tomato Fruit SOD Enzyme Extract

Extract concentration SOD enzyme (%)	Abs 1	Abs 2	Abs 3	SD	Average	%SOD
Tomato 0-20	0,063	0,065	0,064	0,001	0,064	54,32
Tomato 20-40	0,059	0,056	0,056	0,002	0,057	65,90
Tomato 40-60	0,054	0,053	0,052	0,001	0,053	72,99
Tomato 60-90	0,051	0,049	0,049	0,001	0,050	77,83

Table 2. Results of % Inhibition of SOD Activity Test of Tomato Fruit SOD Enzyme Extracts

Extract concentration SOD enzyme (%)	%Inhibition 1	%Inhibition 2	%Inhibition 3	SD	Average
Tomato 0-20	56,14	52,63	54,18	1,76	54,32
Tomato 20-40	63,16	66,67	64,91	2,45	65,90
Tomato 40-60	70,18	73,68	71,93	2,52	72,99
Tomato 60-90	75,44	78,95	79,11	2,07	77,83

Activity testing of the crude extract of SOD enzyme from tomato was performed qualitatively by colorimetric method using SOD assay kit WST-1 from Biovision. The extract was tested to determine its ability to produce SOD enzyme, which is vital in inhibiting superoxide that increases due to photoaging of the skin. Measurement of SOD activity was performed with a microplate reader at a wavelength of 450 nm, producing WST-1 formazan as an indicator of enzyme activity. The results showed that ammonium sulfate precipitation at 90% concentration gave the highest SOD activity, reaching 77.83%. This indicates the potential of tomato fruit SOD enzyme extract as an effective anti-aging agent in protection against collagen degradation by ROS in fibroblasts.

Testing Antiphotaging Activity on 3T3 Fibroblast Cells in-Vitro***Preparation of 3T3 Fibroblast Cells.***

Fibroblast cells are one of the main components that make up the skin hypodermis. The preparation of 3T3 fibroblast cells is done to get enough cell stock to be used as samples. 3T3 fibroblast cells were cultured in-vitro to obtain homogeneous cell samples, so as to provide consistent results on the treatment given. The results of in-vitro culture of 3T3 fibroblast cells can be seen in the figure below.

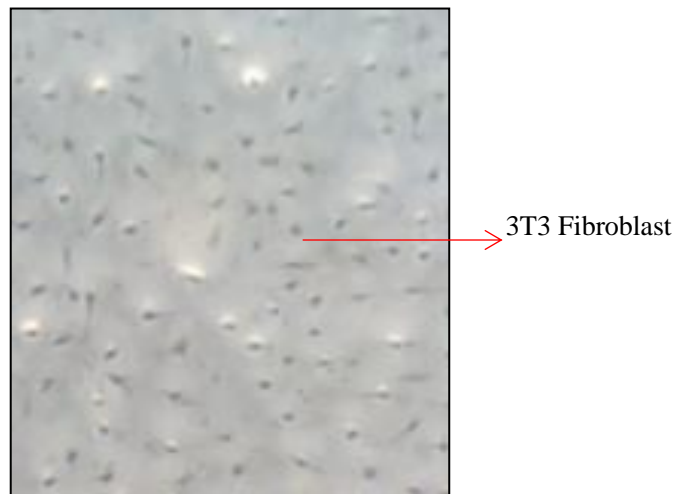


Figure 1. 3T3 fibroblast cells

Figure 10 shows the normal condition of 3T3 fibroblast cells, which are fusiform with branched cytoplasm and fiber-like cell ends. Exposure of 3T3 fibroblast cells to Ultraviolet A (UV A) light aims to create damaged cell samples with decreased collagen deposition, which is indicative of photoaging due to UV exposure. This photoaging is influenced by factors such as the level of UV exposure, the amount of melanin, and the geographical conditions in which individuals live. The results of UV A exposure showed that fibroblast cells had decreased viability and collagen deposition, due to cell damage induced by free radicals from UV A light. This study highlighted that superoxide dismutase (SOD) enzyme from tomato extract, at a certain concentration such as 100 mg/ml, was able to significantly restore fibroblast cell viability, showing a positive impact on collagen deposition. This treatment illustrates that SOD from tomato extract can be potentially an effective antioxidant agent in reducing the adverse effects of photoaging on the skin.

Result Analysis

The results of descriptive analysis showed variations in average fibroblast cell viability and collagen deposition based on the treatment of tomato SOD enzyme crude extract concentration. Cell viability showed the highest average in the 100 mg/ml SOD treatment with a value of 231.25%, while collagen deposition reached the highest average in the same treatment with a value of 147.67%. Data normality test showed that both variables were normally distributed ($\text{sig} > 0.05$), while homogeneity test showed that the variance was homogeneous ($\text{sig} > 0.05$). Anova test showed that variation in concentration of tomato SOD enzyme crude extract significantly affected the decrease in collagen deposition and increase in fibroblast cell viability after exposure to ultraviolet A light ($\text{sig} < 0.05$). Dunnett and Tukey tests showed that most treatments had significant differences compared to the control, with 5 mg/ml ascorbic acid showing better effectiveness in increasing collagen deposition than 100 mg/ml SOD enzyme.

Discussion

Antioxidant activity of tomato (Solanum lycopersicum) fruit SOD enzyme.

The results showed that tomato SOD enzyme activity increased at higher concentrations of crude extract. At the lowest extract concentration of 20%, the SOD activity had reached 54.32%. While at the highest concentration of extract, 90%, the SOD enzyme activity reached 77.83%. These results are in accordance with Baharvand (2015) which explains that high SOD enzyme activity indicates the reduction of superoxide anions associated with xanthine oxidase (XO) activity, and is inhibited by SOD. Free radicals or superoxide produced will react with WST-1 to produce yellow WST-1 formazan dye (Prasetyorini et al., 2014). SOD activity is seen through the degree of inhibition of color formation at the measurement wavelength (450 nm).

The results of this study are also in line with what Saraswati (2016) stated regarding endogenous antioxidants. SOD is one example of natural exogenous antioxidants that are enzymatic and can be obtained from tomato fruit extraction (Stojiljković et al., 2014). Prasetyorini et al (2014) also explained that the presence of antioxidants for the body is an important substance that can inhibit oxidation reactions by binding free radicals and highly reactive molecules. Antioxidants are defined as compounds that have a molecular structure that can provide electrons to free radical molecules without disturbing their function and can break the chain reaction of free radicals (Ministry of Health and Social Welfare of RI. 2000). Antioxidants are useful in preventing the occurrence of various diseases such as cardiovascular disease, coronary heart disease, cancer, and premature aging (Febriyanti, 2013; Ramadhan, 2015).

The SOD enzyme activity of the research results at 20% extract level was able to produce an activity of 54.32%. This is an indicator that the SOD enzyme obtained from tomatoes works well. This is in accordance with Vaya and Aviram (2001) who explained that antioxidants are compounds in low levels that can inhibit the oxidation of target molecules so that they can fight or neutralize free radicals. SOD enzyme as an antioxidant in the chemical sense is a compound that gives electrons, while in the biological sense is defined as a compound that can reduce free radicals and *Reactive Oxygen Species*. SOD enzyme as an antioxidant has a molecule that neutralizes free radicals by accepting or giving electrons to eliminate unfavorable conditions. This shows that the SOD enzyme acts as a radical in the neutralization process of free radical molecules. However, SOD enzyme radicals are more reactive than the free radicals to be neutralized. This SOD enzyme radical can be neutralized by other antioxidants or by other mechanisms that stop the radical. SOD enzymes reduce the speed of initiation reactions in the chain reaction of free radical formation in very small concentrations, namely 0.01% or even less.

SOD enzymes as antioxidants work by catalyzing the change of superoxide anion into hydrogen peroxide which is then converted into water and oxygen by CAT or GPx. The activity of GPx towards H_2O_2 is high compared to CAT due to its kinetic differences. GPx reduces H_2O_2 using glutathione (GSH) as a hydrogen donor. In addition, GPx reduces lipid peroxides (ROOH) to stable and non-toxic fatty acid hydroxyl (ROOH). GPx together with phospholipases convert

phospholipid hydroperoxides (PL-OOH) to phospholipid hydroxides (PL-OH) (Mueller et al., 1997).

SOD is a primary enzyme in the body because it is able to protect cells in the body from free radicals. Fibroblast cell damage can also be triggered by molecules containing oxygen atoms that can produce free radicals or are activated by radicals such as hydroxyl radicals, superoxide, and hydroxy peroxide (Calleja-Agius J and Brincat M. 2013). The main damage to cells occurs due to changes in macromolecules such as fatty acids in membrane lipids, proteins and DNA.

SOD Enzyme Activity in Enhancing Fibroblast Cell Viability.

Skin aging can occur intrinsically and extrinsically. Extrinsic skin aging, also known as premature aging, is intrinsic aging that is exacerbated by exposure to environmental factors such as pollution, nicotine, repetitive muscle movements, various lifestyle components such as diet, sleeping position and overall health, and especially by the effects of chronic exposure to SUVs. Skin aging due to chronic exposure to SUVs (*photoaging*) is mainly characterized by elastosis, which clinically appears as yellowish discolorization and uneven skin surface. Histologically, elastosis appears as a tangled pile of degraded elastic fibers and an amorphous mass composed of disorganized tropoelastin and fibrillin in the dermis. In addition, there is an increase in the amount of basic substances consisting mainly of glycosaminoglycans and proteoglycans, and a decrease in collagen deposition.

Photoaging increases with UV-A and UV-B exposure and is characterized by decreased fibroblast cell viability. The resulting ROS can damage skin DNA. When UV-B contacts the skin, it damages connective tissue and activates protein (AP)-1 and *nuclear factor* (NF)-kB to increase metalloproteinase enzymes containing collagenase, stromelysin and gelatinase in the epidermis and dermis. These enzymes also work to inhibit collagen and elastin resulting in wrinkles.

The results showed that fibroblast cells can increase their viability up to 233% with 100 mg/ml SOD enzyme activity. The decrease in cell viability was observed with the characteristic of black color in the observation preparation. This indicates the number of fibroblast cells that experience death after being exposed to UVA light. Fibroblast cell viability increased with the observation of a round cell shape with fibers at the ends after being treated with SOD enzyme. The results of this study are in accordance with Kumalaningsi (2007) who revealed that tomato fruit contains chemical compounds that are efficacious to prevent various degenerative diseases from various types of cancer such as photoaging, prostate cancer, breast cancer, lung cancer, bladder cancer, cervical cancer, diabetes mellitus, asthma, atherosclerosis, immune function, and heart disease. The prevention of degenerative diseases is due to the chemical compounds contained in tomatoes.

Fresh tomato fruit extract not only contains SOD enzymes, but also has other contents such as lycopene 3.1 - 7.7 mg in every 100 g (Fessenden and Fessenden, 1986; Tonucci et al., 1995). Tomato fruit extract is also rich in vitamins. In every 100 grams of fresh fruit contains vitamin C of 34.38 mg, vitamin A of 112140 IU, vitamin E of 0.68 mg and vitamin B3 of 1.13 mg (Dewi & Naufal, 2011). Tomatoes also contain flavonoid compounds that are very useful for the body, in every 100 g of fresh tomatoes, there are 0.38 mg of flavonoid QE (Eveline et al., 2014).

Decreased fibroblast cell viability is the cause of the aging phenomenon. Aging is a complex process characterized by physiological changes in all organs of the body including the skin in an organism due to a decrease in biological function and the ability of an organism to deal with metabolic stress over time. The skin aging process is divided into two parts: intrinsic (chronological) aging and *photoaging*. Intrinsic aging inevitably occurs in all parts of the skin and causes functional skin loss (Bogor Agricultural University). Intrinsic aging includes clinical, histological and physiological changes that appear in the skin with the passage of time, and is influenced by genetic factors.

Increased fibroblast cell viability by SOD enzyme activity can slow down the skin aging process that occurs naturally according to internal and external aging, which is heavily influenced by the environment. Internal aging such as chronological-aging, biological-aging (genetic), catabolic-aging (chronic diseases, carcinoma), and hormonal-aging. External aging such as photoaging (UV radiation), environmental-aging, mechanical-aging, behavioral-aging, and gravitational-aging. The *stochastic* theory explains that skin degeneration is influenced by cell damage due to environmental factors. Sunlight causes most of the damage due to the formation of ROS that interfere with the formation of biomolecular structures in the skin such as changes in DNA and protein structures.

SOD Enzyme Activity in Enhancing Collagen Deposition

Collagen is the main component of connective tissue and extracellular proteins in the human body (Ganceviciene et al., 2012). There are currently 28 different types of collagen, with collagen types I and III being the main components in the dermis of human skin, which provide the strength of the merchandise space, allowing the skin to function as a defense organ against external trauma (Uito et al., 2008).

UV A light causes damage in the form of decreased collagen deposition in fibroblast cells. The results showed that after receiving radiation for 55 minutes, fibroblast cells experienced collagen deposition. UV A radiation conditions cause damage to fibroblast cell DNA, which has an impact on cell proliferation continuously so that it becomes the beginning of cancer formation. These adverse effects arise due to oxidative stress that occurs after UV light buffering. Oxidative stress is the result of an imbalance between prooxidants (reactive oxygen species) and antioxidants (Sari, 2015).

The results showed that SOD enzyme at a concentration of 100 mg/ml was able to increase collagen deposition up to 147.67% of fibroblast cells that had been buffered by UV A. These results were close to the ability of 5 mg/ml ascorbic acid which reached 176.33%. This indicates that tomato fruit can be used as an ingredient for SOD enzyme in repairing fibroblast cell damage due to UV A radiation. Lyons and Brien (2002) explained that the damage caused by UV A light buffering is called photoaging. Photoaging is a *chronicultraviolet* (UV) superposition that induces changes in skin structure. Manifestations of photoaging include wrinkles in the upper and lower layers of the skin epidermis, development of rough texture, atrophy, and *dyspigmentation*.

The reality of daily life shows that the amount of skin damage caused by sunlight is determined by the amount of radiation buffering and pigment protection a person has. Sun-induced epidermal changes include epidermal thinning and expression of lesions that trigger the activation of keratoses, basal cell carcinoma and squamous cell carcinoma (Arsiwala et al., 2013). Photoaging is characterized by uneven pigmentation, dry, rough, pale, wrinkled skin and decreased strength and elasticity (Lyons and Brien., 2002). In terms of histological studies, photoaging causes the dermis layer to be covered by amorphous masses, disorganized collagen fibers, dilated blood vessels, abnormalities and decreased melanocytes (Walterova et al., 2006).

The positive results of SOD enzyme activity in increasing collagen deposition in this study are in accordance with Lee et al (2004). Lee et al (2004) suggested that the effects of UV radiation can be overcome by SOD. SOD is a preventive antioxidant enzyme, which is a metalloenzyme antioxidant. The mechanism of action of the SOD enzyme in protecting cell damage by converting superoxide anion. Hydrogen peroxide in the mitochondria will be detoxified by the enzyme catalase into compounds H_2O and O_2 , while H_2O_2 that diffuse into the cytosol will be detoxified by the enzyme glutathione peroxidase (Draelos, 2021).

The results of this study also show that fresh tomato fruit has a fairly high lycopene content. The potential of lycopene as an antioxidant and free radical scavenger and inhibitor of singlet oxygen oxidation is a very beneficial effect on human health (Angel, P., et al 2001). Lycopene can also interact with Reactive Oxygen Species (ROS) such as H_2O_2 and NO_2 (Lu et al., 1995; Woodall et al., 1997). Andayani et al (2008) stated that SOD can increase collagen deposition. SOD in methanol extract of tomato fruit has the ability to reduce DPPH free radicals smaller than vitamin C (Kumar et al., 2004). Red tomato fruit contains a lot of vitamin A, vitamin C, minerals, fiber, phenolic compounds and carotenoids (Soehardi, 2004; Tugiyono, 2006). Previous research on tomatoes (*Solanum lycopersicum*) conducted by Sunil Kumar et al., (2004) entitled *Partial Purification and Characterization of Superoxide Dismutase from Tomato (Solanum lycopersicum) Fruit* showed that crude extract of SOD enzyme from tomato fruit amounted to $5.6 \text{ units mg}^{-1}$, and purification of ammonium sulfate amounted to $23.7 \text{ units mg}^{-1}$.

Aging occurs because this sunlight buffering (photoaging) is further increased by UV-A and UV-B buffering. ROS that arise can damage skin DNA. UV-A light in direct contact with the skin will damage connective tissue and activate protein (AP)-1 and *nuclear factor* (NF)-kB to increase metalloproteinase enzymes containing collagenase, stromelysin and gelatinase in the epidermis and dermis. These enzymes also work to inhibit collagen and elastin resulting in wrinkles (Limpiangkanan et al., 2010).

The mechanisms of skin aging caused by intrinsic and extrinsic factors are different. Disturbances in intrinsic factors result in an increase in free radicals and telomere shortening which will lead to a decrease in collagen production. Extrinsic factors (UV light and smoking) cause abnormal elastin growth (Sadick SN et al., 2009). An intrinsic factor that causes an increase in free radicals is obesity. Obesity is a condition of excess or abnormal accumulation of fat tissue. Obesity results in

inflammatory reactions that will increase oxidative stress and telomere shortening (Tzanetakou et al., 2012).

The results of this study are also in accordance with the research of Andayani et al (2008), which states that methanol extract of tomato fruit has the ability to reduce DPPH free radicals smaller than vitamin C. In this study it is also known that fresh tomato fruit has a fairly high lycopene content. The potential of lycopene as an antioxidant and free radical catcher and inhibitor of singlet oxygen oxidation is a very beneficial effect on human health. Lycopene can also interact with Reactive Oxygen Species (ROS) such as H₂O₂ and NO₂ (Lu, et al., 1995; Woodall et al., 1997). Red tomato fruit contains a lot of vitamin A, vitamin C, minerals, fiber, phenolic compounds and carotenoids (Soehardi, 2004; Tugiyono, 2006). Previous research on tomato fruit (*Solanum lycopersicum*) was conducted by Sunil Kumar et al., (2004) entitled *partial purification and characterization of superoxide dismutase from tomato (Solanum lycopersicum) fruit* showed that crude extract of SOD enzyme from tomato fruit amounted to 5.6 mg units⁻¹, and purification of ammonium sulfate amounted to 23.7 mg units⁻¹.

Increased collagen deposition can be understood in skin fibroblast cells that produce procollagen. Under physiological conditions, procollagen is in *soluble* form. *Procollagen* will then be secreted into the extracellular space of the dermis, undergoing an enzymatic process to form a *triple helix* configuration. The *triple helix* will then bind with other extracellular proteins, such as leucine-rich proteoglycans, to form an ordered fibrillar structure, which is referred to as the fibrillogenesis process. The end product of fibrologenesis is the deposition of *insoluble* collagen, which is responsible for the strength and suppleness of the skin (Rittie & Fisher, 2002).

Collagen content per unit skin surface area decreases by $\pm 1\%$ per year during adulthood, and the remaining collagen fibrils appear irregular, dense and granular, and show increased collagen *cross-links* due to decreased synthesis of collagen types I and III, and increased collagenase levels. These changes in collagen lead to increased skin stiffness and wrinkles (Talwar et al., 1995; Yaar & Gilchrist., 2008). Wrinkled, dry rough, saggy skin, as well as the onset of several skin neoplasms are the main changes in the appearance of aging skin (Yaar & Gilchrist, 2008).

UV light can cause a decrease in collagen deposition either through decreased synthesis or increased collagen degradation. Decreased collagen synthesis occurs because UV light buffering of the skin will cause the formation of ROS, which then activates receptors on the cell surface (Xu et al., 2006). These receptors stimulate *mitogen-activated protein* (MAP) kinase and amino terminal kinase (Fisher et al., 1998), thus inducing the transcription of *activator protein* (AP)-1 (Fisher et al., 2002). *Reactive oxygen species* that are formed also damage lipids in the cell membrane, causing the release of ceramide, then activating AP-1 (Yaar & Gilchrist, 2007). Research conducted by Saliou et al (1999) showed that UV exposure to keratinocyte cell cultures starting at a dose of 75 mJ/cm² can increase AP-1 activation.

Increased transcription and activation of AP-1 will decrease the number of receptors and inhibit the effect of *transforming growth factor* (TGF)- β (Karin et al., 1997). The number of TGF- β receptors is also negatively regulated by UV

exposure to fibroblasts that induce *cysteine-rich* protein 61 (CYR61) (Quan et al., 2006), while the effect of TGF- β can also be impaired by SUV exposure that induces Smad7 protein (Quan et al., 2001). *Transforming growth factor- β* plays a role in the transcription of collagen-encoding genes, so a decrease in the number of receptors and disruption of TGF- β effects will decrease the synthesis of type I and III procollagen (Fisher et al., 2002).

CONCLUSIONS

Based on the data obtained and its presentation, it can be concluded that SOD enzyme from *Solanum lycopersicum* can be extracted with varying concentrations, reaching 54.32% from 20% enzyme extract, 65.90% from 40% enzyme extract, 72.99% from 60% enzyme extract, and 77.83% from 90% tomato fruit SOD enzyme extract. This SOD enzyme was also shown to be able to increase fibroblast cell viability and collagen deposition on exposure to Ultraviolet A light, with collagen deposition reaching 147.67% and cell viability reaching 231.25% at an SOD enzyme concentration of 100 mg/ml. The EC90 value for fibroblast cell viability was 29.94% and for collagen deposition was 51.79%. These results show the significant effect of SOD enzyme concentration in increasing viability and collagen deposition in fibroblast cells undergoing photoaging effect. In this case, it is suggested the need for further purification of SOD enzyme from tomato fruit extract in future studies to evaluate its effect on photoaging treatment by UV A light on fibroblast cells. Product development from natural materials in the form of tomato fruit SOD enzyme extract isolate is also suggested as an anti-aging potential, and can be a reference in the discovery of active compounds as anti-aging from natural materials.

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