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# Collagen Deposition Effect of Superoxide Dismutase Corn Kernel Extract (*Zea mays* L.) as Skin Photoaging Treatment

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**Abstract:** Intensive exposure to UV-A rays can develop photoaging in human skin. To aid this condition, several potential anti-photoaging mechanisms that have been investigated can be implemented, including collagen promotion. Collagen production in the skin has been studied promoted by an enzymatic antioxidant, superoxide dismutase (SOD). Here, we found out the collagen deposition effect from SOD crude extract of corn kernel in 3T3 fibroblast cells as alternative ingredients of anti-photoaging. Soluble protein content and SOD activity assay were executed first to prop up the main test. Collagen deposition effect was measured using staining method with picric acid solution. The result showed the highest Soluble protein and SOD activity was in 100% precipitation of corn kernel SOD extract (20.91 mg/ml and 65.50% respectively). The highest collagen deposition percentage obtained was 106.7% in 100 mg/ml corn kernel SOD extract. The collagen deposition percentage obtained was not significantly different from 2.5 mg/ml ascorbic acid. Based on viewing collagen condition under a microscope, there was an improvement in collagen, which was indicated by the interconnected collagen fibers when the extract and ascorbic acid were given. The research that has been conducted concludes that corn kernel SOD extract has anti-photoaging activity through collagen deposition effect.

**Keywords:** Photoaging; Collagen; Corn; Fibroblast; Superoxide dismutase

## 1. INTRODUCTION

Mechanisms that cause intrinsic aging include ROS, cellular metabolism, genetics, and hormonal changes [1] while several factors, such as environmental pollution, intensive sun exposure, bad diet patterns, and smoking habits, can cause extrinsic aging. Aging is related to symptoms like coarse wrinkles, loss of elasticity, sagging, and a rough appearance. In young skin, synthesis and degradation of collagen are balanced, so there will be no significant symptoms of aging. Gradually, when the function of cells in skin is weakened or damaged, aging is accelerated by drastic changes in fibroblast senescence, failing collagen and elastin recovery, epidermal thinning, reduced blood vessel density, and other damage[2]. Since 1969, it has been suggested that prolonged exposure to ultraviolet (UV) radiation from the sun is a significant contributor to extrinsic skin aging, also known as photoaging[3]. The prevalence of pathologically modified elastic fibers is the most prominent histological characteristic of photoaging. MMP activation, which also describes the breakdown of collagen fibers, is the primary source of elastic fiber structure deterioration in photoaging. In photoaged skin, not only the density of elastic fibers reduced, but also the synthesis of new elastic

fibers is reduced. The last, ECM decreases and the breakdown of collagen fibers increases, that caused by increasing hyperplastic/activated fibroblast and MMP levels, and increasing inflammatory cells such as eosinophils, lymphocytes, mast cells, and mononuclear cells in photoaged skin [4].

The skin thins and loses a significant amount of collagen when it ages. The main harmful alteration seen in aging skin is the breakdown of collagen. The activation of matrix metalloproteinases (MMPs) exacerbates the breakdown of collagens. By binding to antioxidant response sites and reducing MMP-1 production, Nrf2, a master transcription factor for the cellular antioxidant regulator, contributes significantly to photo-oxidative damage. There have also been reports that MMP-1 expression in human dermal fibroblasts (HDFs) is significantly influenced by the activation of adenosine monophosphate-activated protein kinase (AMPK). Oxidative stress also directly interferes with the TGFβ pathway, which lowers the formation of collagen [2].

Based on the wavelength and energy carried, there are three types of UV radiation, namely UVC (100-280 nm), UVB (280-315 nm) and UVA (315-400 nm) [5]. Due to their high energy, UVA photons can enter the skin's dermis layer, and their effects are now known to have a role in the development of melanoma skin cancer and premature skin aging [6]. The quantity of energy absorbed by base pairs in the DNA chain is linked to the direct damaging mechanism of UVB and UVA action on DNA molecules [7]. The release of ROS components is the primary mechanism by which UV exposure stimulates molecular responses. At elevated quantities, these extremely reactive substances—which include superoxide anions, peroxides, and singlet oxygen—can change the characteristics of collagen and elastin, the two primary proteins that comprise the skin [8].

Excess free radicals in the body due to UV exposure can be neutralized by antioxidant compounds. Antioxidants are those produced naturally in the body (endogenous antioxidants) such as superoxide dismutase enzymes, glutathione peroxidase, and catalase as well as antioxidants obtained from outside the body (exogenous antioxidants) such as vitamins E and C, minerals (manganese, zinc and selenium), and other antioxidant compounds such as flavonoids and beta-carotene [9]. Three human superoxide dismutase (SOD) isoenzymes have been identified: extracellular SOD (EC-SOD), mitochondrial manganese SOD, and copper-zinc SOD, which is found in the cytoplasm and nucleus[10]. Research has indicated that SOD protects type I and type IV collagens from oxidative damage and prevents collagen-mediated injury by interacting with them via the heparin and/or matrix binding domain[11].

SOD enzyme extraction has been carried out on various natural materials such as plants, animals and bacteria. SOD in plants has been extracted from corn plants [12], [13]. Corn plants have begun to be intensively planted in order to achieve food self-sufficiency in Indonesia [14]. Based on data from the 2013 Agricultural Census (ST2013), corn plants are the most widely grown secondary crops in Papua province, namely 65,605 household horticultures with a planting area of 38,533,447 ($m^2$[15]. Scientists and researchers study medicinal plants to identify chemical compounds that have pharmacological activity. By understanding these compounds, they can develop more effective natural medicines [16]. Previous studies on SOD extraction from corn kernels and testing of anti-photoaging activity have been carried out with fibroblast cell viability parameters [13]. This study aimed to find out the collagen deposition effect from SOD crude extract of corn kernel in 3T3 fibroblast cells as alternative ingredients of anti-photoaging.

## 2. MATERIALS AND METHODS

### *2.1. Equipment and Materials*

The equipment used in this study were knives, blenders (Philips; Jakarta, Indonesia), plastic containers, analytical scales (Fujitsu FS-AR; Kawasaki, Japan), beakers (Iwaki Pyrex; Sumedang, Indonesia), Erlenmeyer flasks (Iwaki Pyrex; Sumedang, Indonesia), pasteur pipettes (OneMed; Sidoarjo, Indonesia), iron spatulas, magnetic stirrer bars (Vitlab; East Jakarta, Indonesia), hot plate stirrer C-Mag HS 7 (IKA; Selangor, Malaysia), pH meter HI5221 (Hanna; Jakarta, Indonesia), centrifuge type CE146 (Biocen; Dobern, Germany), conical tubes (Fisherbrand; Pittsburgh, Pennsylvania), conical tube racks (Fisher; Pittsburgh, Pennsylvania), dialysis clips, micropipettes (MettlerToledo; Bekasi, Indonesia), blue tip (MettlerToledo; Bekasi, Indonesia, yellow tip (MettlerToledo; Bekasi, Indonesia, vortex SI-0286 (GENIE2; Jakarta, Indonesia), micro tube, microplate reader (Fisher; Pittsburgh, Pennsylvania), 96 well microplate (Iwaki; Sumedang, Indonesia), $CO_2$ incubator Ico 150 (Memmert; Pune, India), Laminar Air Flow (Robust; Singapore), Inverted Microscope DX2058PLPOLRI Trinocular microscope (Euromex; Duiven, Netherlands).

The materials used in this study were corn kernels, $NaOCl^-$ (sodium hypochlorite) 2.5% (OneMed; Sidoarjo, Indonesia), aquabidest, $KH_2PO_4$ (potassium dihydrogenphosphate) (Merck; Darmstadt, Germany), $K_2HPO_4$ (dikalium phosphate) (Merck; Darmstadt, Germany), $(NH_4)_2SO_4$ (ammonium sulfate) (Merck; Darmstadt, Germany), dialysis tube (Wards Science; West Henrietta, New York), CBB G-250 (Coomasie Brilliant Blue G-250) (Solarbio; Beijing, China), BSA (Bovine Serum Albumin) (Merck; Darmstadt, Germany), Bradford Reagent (Sigma-Aldrich; St. Louis, Missouri), ((superoxide dismutase activity assay kit (Sigma-Aldrich; St. Louis, Missouri), fibroblast cells 3T3, NaHCO3 (sodium bicarbonate) (Merck; Darmstadt, Germany), HCl (hydrogen chloride) 1 N (Merck; Darmstadt, Germany), NaOH (sodium hydroxide) 1 N (Merck; Darmstadt, Germany), penicillin-streptomycin (Hebei Veyong Pharmaceutical Co. Ltd.; Hebei, China), DMEM (Dulbecco's Modified Eagle's Medium) (HiMedia Laboratories Pvt. Ltd.; Thane, Maharasthra), PBS (Phosphate Buffer Saline) 1X (PluriSelect; Leipzig, Germany), pure ascorbic acid (Merck; Darmstadt, Germany), sirius red, picric acid (Pallav Chemical & Solvents Pvt. Ltd.; Mumbai, Maharasthra), tissue (Nice; Makassar, Indonesia), aluminum foil (Klin Pak; Bogor, Indonesia).

### *2.2. Research Procedure*

#### 2.2.1. Enzyme Extraction from Corn Kernels

Superoxide dismutase enzyme extraction from corn kernels was carried out using centrifugation, precipitation and dialysis methods [13]. The precipitation concentrations made were 40%, 60%, 80% and 100%. This concentration was chosen regarding to the prior study [13].

#### 2.2.2. Measurement of Soluble Protein Content

a. Making Bradford reagent

CBB G-250 (Coomasie Brilliant Blue G-250) in the amount of 10 mg was dissolved in 5 ml of 95% ethanol. 85% phosphoric acid in the amount of 10 ml was added to the solution and diluted with distilled water to 100 ml[17].

b. Making a standard protein solution

BSA (Bovine Serum Albumin) in the amount of 100 mg was weighed, then dissolved with 10 ml of distilled water to make a 1% BSA standard solution[17].

c. Making a standard curve

Seven test tubes were prepared to make standard solutions. The composition of the solution can be seen in Table 1.

**Table 1.** Composition of BSA standard solution

| Concentration (mg/ml) | Solvent (µl) | Bradford (µl) | BSA (µl) |
|---|---|---|---|
| 1 | 799 | 200 | 1 |
| 2 | 798 | 200 | 2 |
| 4 | 796 | 200 | 4 |
| 8 | 792 | 200 | 8 |
| 16 | 784 | 200 | 16 |
| 32 | 768 | 200 | 32 |

The absorbance of each solution was measured using a UV-Vis spectrophotometer at a wavelength of 595 nm. The curve between the absorbance value and concentration was made to obtain a linear regression equation[18].

d. Measurement of dissolved protein levels

A sample of 2 µl was added to 200 µl of Bradford solution and then made up to a volume of 1 ml with distilled water. The absorbance was measured at a wavelength of 595 nm. The absorbance value obtained was entered into a linear regression equation to obtain the soluble protein levels.

2.2.3. SOD Activity Assay

Antioxidant activity testing was carried out using the WST-1 method on 40%, 60%, 80% and 100% precipitation corn kernel SOD extract [13].

2.2.4 Collagen Deposition Effect Test

Anti-photoaging activity testing was carried out using collagen deposition parameters. The stages of making liquid media, making culture media, preparing 3T3 fibroblast cells, exposing 3T3 fibroblast cells to UV-A rays and making a stock solution of corn kernel SOD extract can be seen in the study [13].

Determination of collagen deposition effect was carried out by removing the media in the plate that had been exposed to UVA light, then the test solution was put into a plate containing cells and incubated for 24 hours. After the incubation process, the solution in the plate was discarded. The cells in the plate were washed 3 times using 100 µl of 1X PBS. The cells were fixed using 100 µl of cold methanol and incubated for 2 hours in a 5% $CO_2$ incubator. Cold methanol was discarded and cells were stained with 100 µl of PSR solution, then incubated for 10 minutes in a 5% $CO_2$ incubator. The PSR solution was discarded and cells were washed with 100 µl of 0.1 N HCl solution. The collagen condition in the cells was observed under an inverted microscope. The HCl solution in the plate was discarded then 100 µl of 0.5 N NaOH solution was added, and the collagen deposition absorbance value was read on a microplate reader using a wavelength of 595 nm. This research was conducted

in the Research Center for Biotechnology, the Laboratory of Dermatology and Venereology and the Laboratory of Parasitology of Gadjah Mada University.

*2.3. Data Analysis*

Protein levels and SOD activity SOD were analysed using Microsoft Excel software. The effect of collagen deposition of SOD extract from corn kernels was analysed using One Way ANOVA which was first tested for data normality. Normal data is indicated by a significance value of more than 0.05 if abnormal data is obtained with a significance value of less than 0.05. The difference test is carried out using a non-parametric test. The difference test is carried out by first testing the homogeneity of the data, where homogeneous data is indicated by a significance value of more than 0.05. In contrast, inhomogeneous data is indicated by a significance value of less than 0.05. If the data is homogeneous, then the Tukey post hoc test will be used. If the data is not homogeneous, then Tamhane's T2 test is used.

## 3. RESULTS AND DISCUSSION

*3.1. SOD Extraction*

The purpose of enzyme extraction is to separate enzymes from cells/tissues found in bacteria, animals and plants [19]. Before being extracted, corn kernels are sterilized first. This process aims to reduce contamination that may be present on the surface of the plant. The surface of the plant is exposed to the environment (air, soil and other sources), so there is potential for contamination [20]. The sterilizing agent that was used $NaOCl^-$. The enzyme extraction process begins with crushing corn kernels using a blender. This destruction aims to lyse the cells. SOD is available in 3 types, namely the SOD 1(Cu/Zn SOD) present in cytoplasm, the SOD 2 (Mn SOD) is present in mitochondrial matrix, and SOD 3 is present in extracellular. So, this step is advantage to extract even the intracellular SOD (SOD 1 and 2) and the extracellular SOD (SOD 3). Potassium phosphate buffer used in SOD extraction to prevents denaturation or aggregation of the protein.

The next process is centrifugation. This process aimed to separate the supernatant (the soluble part) and debris (the insoluble part)[21], where the enzyme is found in the supernatant. This happens because protein molecules have a smaller density than cells, so the enzymes are found in the soluble part. This supernatant part is called the crude enzyme extract which will be further processed in the partial purification of the SOD extract.

The next process is enzyme purification using the precipitation and membrane filtration method. Precipitation is a straightforward and cost-effective method for purifying proteins, often applied in the initial stages of the process. The most commonly used precipitation agents include ammonium sulfate and organic solvents like ethanol or acetone. Ammonium sulfate is frequently employed to precipitate proteins selectively, depending on their solubility at varying concentrations. In a solution, proteins form hydrogen bonds with water molecules due to their accessible ionic and polar groups. Proteins with lower solubility are the first to precipitate as the ammonium sulfate concentration rises. This technique allows the extraction of a diverse array of proteins based on their solubility characteristics. The yield of corn kernel SOD extract can be seen in Table 2.

**Table 2.** Results of Corn Kernel SOD Extraction

| Corn Kernel SOD Extract Concentration | Fresh Sample (g) | Extract (g) | Yield (%) |
|---|---|---|---|
| 40 | 2.000 | 4.83 | 0.24 |
| 60 | 2.000 | 5.27 | 0.26 |
| 80 | 2.000 | 5.69 | 0.28 |
| 100 | 2.000 | 6.03 | 0.30 |

The addition of ammonium sulfate to the precipitation process is carried out at low temperatures. Protein precipitation will increase when ammonium sulfate is added at high concentration and low temperature. The higher the concentration of ammonium sulfate added to the crude SOD extract, the more enzymes will precipitate due to the lower solubility of the protein in water. This phenomenon occurs because ammonium sulfate and protein compete for affinity with water molecules. Ammonium sulfate has a higher degree of ionization than protein, so ammonium sulfate has a stronger bond to the water surrounding the protein. The advantage of ammonium sulfate in the precipitation process is that the low density of ammonium sulfate means that ammonium sulfate is not precipitated during enzyme precipitation. Another advantage is that with a large molarity, ammonium sulfate can precipitate many enzymes in the sample [22].

*3.2. Soluble Protein Content*

SOD is an antioxidant enzyme that consists of polypeptides just like other proteins. The quantity of protein is an important metric to measure during protein purification, for calculating yields or the mass balance, or determining the specific activity/potency of the target protein The results of measuring the soluble protein content of corn kernel SOD extract are shown in Table 3.

**Table 3.** Soluble protein content of corn kernel SOD extract

| Corn Kernel SOD Extract Concentration | Soluble Protein Content (mg/ml) ± SD |
|---|---|
| 40% | 3.32 ± 0.004 |
| 60% | 8.15 ± 0.015 |
| 80% | 16.76 ± 0.112 |
| 100% | 20.91 ± 0.011 |

Determination of soluble protein levels was carried out using the Bradford method. The Bradford method is a method of measuring soluble protein levels that involves CBB dye. The principle of this method is based on the formation of a complex between CBB and a protein solution containing amino acid residues with aromatic side chains (tyrosine, tryptophan and phenylalanine) or basic (arginine, histidine and leucine). The bond between the protein and the CBB dye will form a complex through ionic interactions between the positive charge of the protein on the amine group and the sulfonic acid group. Peptides or proteins with a large molecular weight (more than 3000 Da) can produce a blue color using this reagent. The absorbance value is directly proportional to the protein content in the solution because the positive charge of the protein is directly proportional to the number of ligands that bind to the protein molecule [23]. Protein absorbance can be measured

using spectrophotometry at a wavelength between 465-595 nm. Based on the result in Table 3, the higher concentration of the extract showed an increase in soluble protein. These results can lead us to the hypothesis that the higher the concentration of SOD extract, the higher the collagen deposition effect that come up with.

*3.3. SOD Activity Assay*

Based on its SOD activity, the SOD extract of corn kernel ammonium sulfate precipitation with a concentration of 100% was chosen for use in the collagen deposition effect test. The SOD activity of the SOD extract of corn kernel is shown in Table 4.

**Table 4.** SOD activity of corn kernel SOD extract

| **Corn Kernel SOD Extract Concentration** | % Inhibition ± SD |
|---|---|
| 40% | 46.78 ± 5.36 |
| 60% | 52.63 ± 3.51 |
| 80% | 60.23 ± 3.65 |
| 100% | 65.50 ± 5.64 |

WST produces water-soluble formazan dye upon reduction with $O_2^*$. The reduction value of WST-1 from $O_2^*$ is linearly correlated with xanthine oxidase activity, which is inhibited by SOD. Xanthine oxidase activity can be observed using the colorimetric method. The principle of SOD measurement is that free radicals or superoxide produced change WST-1 to produce yellow WST-1 formazan. In addition, SOD works together with WST-1 to react with superoxide, thereby stopping the formation of colored substances [13]. The 450 nm wavelength has the ability to analyze the absorption of the resulting color [24].

*3.4. Collagen Deposition Effect*

The body can express 28 types of collagen. However, only collagen types I and III play a role in skin physiology and histology [25], [26], [27]. Type I collagen plays an important role in providing structural integrity and mechanical resistance of the skin because it is the main part of the fibrillar extracellular matrix, also known as fibrous collagen [28]. The composition of type I collagen fibrils in young skin reaches 85-90% with a long-chain triple helix structure[25], [27], [29]. Type I collagen undergoes structural and organizational changes during aging. They lose ECM protein synthesis, experience increased metalloproteinase degradation, and experience collagen fibril fragmentation. As a result, mechanical strength is reduced [26]. Collagen reduction in aging skin causes wrinkles and loss of elasticity. This is in contrast to young skin, which has intact, abundant, dense, and well-organized collagen fibrils [30]. This condition has led to many studies that had been conducted to find compounds that can increase collagen deposition related to photoaging cases. The percentage of collagen deposition and collagen images from corn kernel SOD extract and normal, negative, and positive controls in 3T3 fibroblast cells after exposure to UV-A rays can be seen in Table 5 and Figure 1.

**Table 5.** Percentage of collagen deposition in 3T3 fibroblast cells

| Sample | % Collagen Deposition ± SD |
|---|---|
| NC | 23.7±1.5[a] |
| NmC | 100.0±0[b] |
| E 12.5 mg/ml | 58.2±5.0[c] |
| E 25 mg/ml | 71.3±2.5[d] |
| E 50 mg/ml | 78.5±1.8[d] |
| E 100 mg/ml | 106.7±2.7[b] |
| PC 1.25 mg/ml | 82.5±2.6[d] |
| PC 2.5 mg/ml | 111.0±5.2[b] |
| PC 5 mg/ml | 176.5±6.4[e] |
| PC 10 mg/ml | 210.8±5.1[f] |

Description:
NC : Negative Control; NmC : Normal Control; E : Corn kernel SOD extract; PC : Positive Control
*Numbers followed by the same letter are not significantly different in the Tukey HSD Test (0.05)

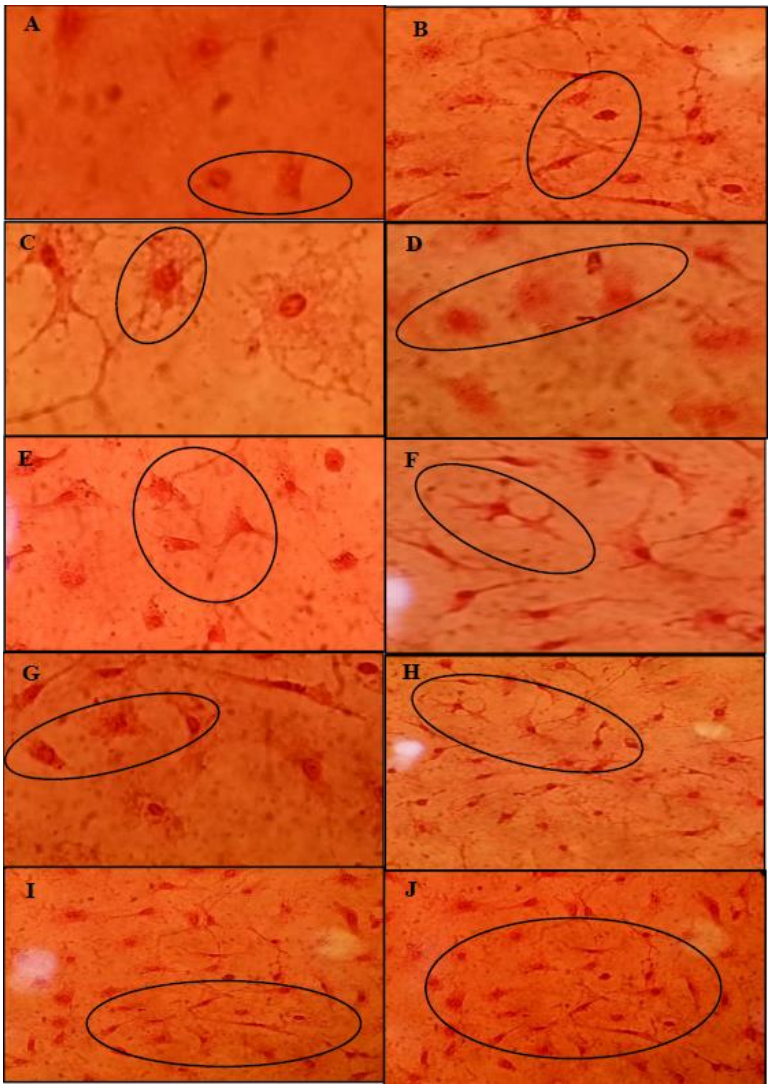


**Figure 1.** Collagen image after exposure to UV-A rays in: Negative control (A); Normal control (B); SOD 12.5 mg/ml (C); SOD 25 mg/ml (D); SOD 50 mg/ml (E); SOD 100 mg/ml (F); Ascorbic acid 1.25 mg/ml (G); Ascorbic acid 2.5 mg/ml (H); Ascorbic acid 5 mg/ml (I); Ascorbic acid 10 mg/ml (J)

The result of collagen deposition analysis showed that the collagen deposition percentage of normal control (did not exposed to UV light), 100 mg/ml corn cernel SOD extract and 2.5 mg/ml ascorbic acid was not signifficantly difference. So we can say that the corn kernel SOD extract with the concentration of 100 mg/ml can repair the damaged collagen because of UV-A rays equivalent to collagen that is not exposed by UV-A rays. Photoaging can occur due to several hypotheses theory, such as the oxidative stress, the inflammatory responses, the matrix metalloproteinase abnormal expression, the hyaluronidase abnormal expression, the elastase abnormal expression and the melanin over-synthesis [31]. According to earlier research, UV exposure can cause skin lesions, decrease skin suppleness, and increase skin dryness in addition to speeding up aging. Both the physiological structure of the tissue and the elastic fibers of mouse skin can be harmed by prolonged UV exposure [32]. UV exposure affects collagen in the skin in two ways, namely decreasing collagen synthesis and increasing collagen degradation through signaling pathways such as mitogen-activated protein kinase (MAPK), NF-Kb (Nuclear Factor-κB), and promoting the development of matrix metalloproteinase (MMP) which causes degradation of the skin extracellular matrix and fibroblast apoptosis, which reduces collagen levels [30]. Activation of the cytokine receptor NF-Kb (Nuclear Factor-κB) and the growth factor receptor TGF-β (Transforming Growth Factor β) on the fibroblast cell membrane can activate ERK (Extracellular Signal-Regulated Kinase), JNK (c-Jun N-terminal Kinase) and p38MAPK. Activation of ERK and JNK/p38MAPK can result in phosphorylation of transcription factors ELK-1 (E-26 Like Protein 1) and c-Jun/Atf-2. Phosphorylation of these transcription factors will activate and increase the expression of AP-1 transcription factors in the cell nucleus [33].

AP-1 activation in fibroblast cells stimulates transcription of genes encoding MMP-1 (collagenase), MMP-3 (stromelysin) and MMP-9 (gelatinase) [34], suppresses procollagen gene expression resulting in decreased collagen synthesis and inhibits the synthesis of transforming growth factor-ß1 (TGF-ß1). The TGF-β1 receptor can induce the synthesis of cysteine-rich protein 61 (CYR61). TGF-β1 plays a role in the transcription of collagen-encoding genes, so inhibition of TGF-β1 synthesis will inhibit the synthesis of pro-collagen types I and III [35], [36], [37].

The increase in the percentage of collagen deposition in the extract group compared to the negative control group was due to the presence of SOD activity in the corn kernel SOD extract. Superoxide dismutase (SOD) acts as an antioxidant enzyme that scavenges oxygen radicals through oxidation/reduction cycles at a very high reaction rate through transition metal ions present at the active site. SOD decomposes $O_2^{\bullet-}$ into $H_2O_2$ with the release of molecular oxygen. SOD is a kind of enzyme containing Cu, Mn, Zn, and other metal ions [11]. The SOD content in corn kernel SOD extract is posibly blocking the receptor activation and expression of AP-1 and MMP-1 transcription factors by reducing the number of SORs in fibroblast cells. SOD activates the collagen synthesis pathway through activation of TGF-β1 and procollagen gene expression [38].

Nuclear factor-erythroid 2-related factor 2 (Nrf2) protects skin cells from UV radiation-induced oxidative damage and cellular dysfunction. The Nrf2/antioxidant response element (ARE) pathway is considered to be one of the principal defense mechanisms against oxidative stress, regulating the expression of multiple detoxification/antioxidant genes, such as heme oxygenase-1 (HO-1), quinone

oxidoreductase 1 (NQO1), CAT, and SOD. When induced by antioxidants, Nrf2 disrupts Kelch-like ECH-associated protein 1 (Keap1), the primary molecule responsible for its negative regulation, which is followed by rapid nuclear translocation and transactivation of ARE-associated genes [**19**]. This process promotes ROS scavenging, maintains redox homeostasis, suppresses inflammation, and repairs damaged DNA, thereby supporting the survival of cells in a pro-oxidant environment [39]. Previous studies have shown the results of increased fibroblast cell viability by corn kernel SOD extract [13]. The increase in fibroblast cell viability will directly affect collagen repair. Collagen is produced by fibroblasts, therefore, hardness and decreased elasticity indicate a decrease in fibroblast collagen production [40].

Measurement of collagen deposition is done by staining collagen with picric acid solution with sirius red. Sirius red is an anionic dye of the azo group with a molecular weight of 1372 which is used to stain collagen in connective tissue and to calculate the amount of collagen in tissue. The sulfonic acid group of sirius red interacts with the positive group of protein is how collagen interacts with sirius red [41].

Pre-treatment with acid results in an increase in weak intermolecular and intramolecular structural bonds in skin collagen proteins due to partial damage to the amino acid bond chains [40]. H+ ions play an important role in breaking intra- and intermolecular collagen bonds through collagen hydrolysis. The higher rate of hydrolysis causes the breakdown of triple helix collagen into larger $\alpha$, $\beta$, and $\Upsilon$. This condition is used in the extraction process, which results in collagen solubility. Continuous hydrolysis can cause shorter collagen molecular chains, so much dissolved collagen is produced in the washing process. Once optimal conditions are met, increasing acid concentration will decrease gelatin yield due to excessive collagen hydrolysis and protein loss in the washing process. Hydrochloric acid requires a lower concentration to achieve optimal extraction conditions compared to other types of acids [42].

## 4. CONCLUSION

The conclusion drawn from the research is that corn kernel SOD extract has anti-photoaging activity based on collagen deposition parameters compared to the control group.

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