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Research Article

Antioxidant Activity of Ethanol Extract of Secang Wood (*Caesalpinia sappan* L.) and Its Effects on Cell Viability and Collagen Synthesis in Blue Light-Exposed Fibroblasts

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ABSTRACT

Exposure to blue light (400-500 nm), derived from sunlight and artificial light sources such as electronic devices and indoor lighting, has been linked to harmful biological effects. Prolonged exposure can reduce antioxidant capacity, leading to oxidative stress, cellular dysfunction, and DNA damage. *Caesalpinia sappan* (secang wood) contains flavonoids and phenolic compounds with known antioxidant properties. This study aimed to evaluate the antioxidant activity of the ethanol extract of Indonesian secang wood and its effects on fibroblast viability and collagen synthesis following blue light exposure. Phytochemical analysis was performed to determine flavonoid and phenolic content, while antioxidant activity was assessed using the DPPH assay. Fibroblast viability was analysed using the MTT assay, and collagen synthesis was measured using the Sirius Red assay. The extract demonstrated high flavonoid (5.93 mg QE/100 g) and phenolic content (2.86 mg GAE/100 g), along with strong antioxidant activity (IC_{50}: 7.8 µg/mL), exceeding that of the reference herbal extract (*Artemisia capillaris*). In fibroblasts exposed to blue light, treatment with secang wood extract significantly improved cell viability and collagen synthesis compared to untreated controls. In conclusion, secang wood ethanol extract exhibits potent antioxidant activity and enhances fibroblast function after blue light exposure.

1. Introduction

Visible light is the spectrum of light that can be seen by the human eye, with wavelengths between 400 and 700 nm (Pourang *et al*. 2022; Ge *et al*. 2023). One of the visible light spectra with high energy is blue light (from 400 to 500 nm) (Ge *et al*. 2023). Blue light spans wavelengths of 400-500 nm, with shorter wavelengths, particularly in the 410-420 nm range, demonstrating greater toxicity. In general, toxicity increases as the wavelength decreases toward 400 nm (Opländer *et al*. 2011). Blue light, whether from the sun or electronic devices, is a significant factor in ageing (Ferreira *et al*. 2021). Prolonged exposure to blue light increases oxidative stress, disrupts the skin barrier, and contributes to inflammation and pigmentation (Avola *et al*. 2018;

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Bacqueville *et al*. 2021; Wortzman and Nelson 2021; Ge *et al*. 2023).

The 60% of the human population uses digital electronic devices intensively for about 6 hours per day. This results in global exposure to blue light with varying sources (Avola *et al*. 2018). The skin is exposed to blue light for long periods each day, which can cause cumulative skin damage (Liebel *et al*. 2012). Accordingly, an in vitro skin model showed increasing molecular damage after exposure to blue light for 1, 2, and 4 hours (Lago *et al*. 2024). Previous research shows that blue light exposure alters normal skin structure and promotes premature aging (Avola *et al*. 2018). Blue light with wavelengths of 410 and 420 nm can increase oxidative stress by reducing the antioxidant properties of fibroblasts without causing toxic effects (Sadowska *et al*. 2021). At a wavelength of 420 nm, blue light significantly inhibits TGF-β signalling, including fibroblast differentiation into myofibroblasts (Krassovka *et al*. 2020).

Skincare products derived from natural materials for protection against the harmful effects of light from increased electronic device use have gained popularity in recent decades (Ferreira *et al*. 2021; Mohd-Setapar *et al*. 2022). This growing demand emphasises the need to explore biodiversity-rich regions as valuable sources of novel natural materials for photoprotection. Indonesia is one of the richest countries in plant biodiversity. Approximately 30,000 plants have been identified. Indonesian plants offer great potential as raw materials for producing herbal cosmetics (Wathoni *et al*. 2018; Batubara and Prastya 2020). Given that exposure to blue light is unavoidable in everyday life, a cosmetic product needs to contain active components that protect the skin from the harmful effects of both ultraviolet and blue light (Ferreira *et al*. 2021).

Recently, the active ingredient in cosmetics marketed for blue-light protection is *Artemisia capillaris* (*A. capillaris*) (Morvan *et al*. 2019). Exposure of fibroblasts to blue light leads to decreased viability, increased reactive oxygen species (ROS) levels, and decreased ATP production (Morvan *et al*. 2019). *Artemisia capillaris* is a herbal plant in China that has long been used for its antipyretic, anti-inflammatory, diuretic, and antioxidant activities (Hong and Lee 2009; Morvan *et al*. 2019). This extract can reduce the negative impact of blue light from computer screens, cellular phones, and digital tablet screens by preserving cellular viability, maintaining ATP levels, and reducing skin contractions, making it a potential active ingredient in some skin care products (Morvan *et al*. 2019). Secang wood/ *Caesalpinia sappan* (*C. sappan*) is known to have natural antioxidant, anti-inflammatory, and antimicrobial properties, as well as antidiarrheal properties. It even maintains skin and heart health by improving blood circulation. Therefore, it can be used as an alternative protective agent against blue light (Ahn *et al*. 2018).

Secang wood is a traditional plant often used as a spice, a medicinal material, or in mixtures for drinks or foods (Setyowati *et al*. 2023). This plant's heartwood is often consumed as a traditional drink, believed to offer various benefits and serve as a cultural identity for each generation (Wu *et al*. 2011; Setyowati *et al*. 2023). Studies have shown that secang wood extract exhibits antimicrobial, antioxidant, anticonvulsive, and anti-inflammatory activities (Tewtrakul *et al*. 2015; Shedoeva *et al*. 2019). Additionally, secang wood can promote cutaneous fibroblast migration and proliferation as well as collagen synthesis, thereby improving wound healing and the skin ageing process (Shedoeva *et al*. 2019).

In this experimental study, our purpose was to assess the phytochemical constituents and antioxidant activity of the ethanol extract of secang wood from Bogor, West Java, Indonesia, as well as its effects on cell viability and collagen synthesis in fibroblasts following a single blue-light exposure at various doses.

2. Materials and Methods

2.1. Study Design and Location

Our study is an in vitro experimental study. The subjects in our study were primary human fibroblasts. The research was conducted at Universitas Gadjah Mada (UGM) in Yogyakarta from September 2022 to September 2023. Sample determination was conducted at Bogor Agricultural University (IPB). The Declaration of Helsinki was followed in conducting the study. The institutional ethics committee approved the protocol, and fibroblasts used in this study were obtained from a healthy human donor with written informed consent. Approval for the study (Ref. No. KE/FK/1237/EC/2022 issued on September 27th, 2022) was obtained from the Medical and Health Research Ethics Committee (MHREC), Faculty of Medicine, Public Health and Nursing Universitas Gadjah Mada - Dr Sardjito Hospital, Yogyakarta, Indonesia.

2.2. Sample Preparation and Extraction

In this study, we use secang wood, a sample with voucher number BMK0120092016, which originated from Bogor, West Java, Indonesia. The secang wood extraction process was done using a modification method (Erwin *et al*. 2022). Before extraction, the heartwood from secang wood stems was sorted and washed with running water, then dried at room temperature (±25°C) for 3 days or in a drying cabinet at 40°C for 2 days. Once the stems were dry and clean, they were pounded and blended into a fine powder. The extraction process for the secang wood involved maceration and evaporation, with 96% ethanol added to 350 g of the secang wood sample until the mixture dissolved. This procedure was carried out three times at room temperature every 24 hours. The resulting filtrate from the maceration process was collected, filtered, and further concentrated by evaporation using a rotary evaporator (Erwin *et al*. 2022). Furthermore, *A. capillaris*, as a reference herbal extract, was obtained from Codif Technologie Naturelle, France (Morvan *et al*. 2019).

2.3. Phytochemical Analysis

A modified phytochemical analysis was conducted based on prior studies by Pandey and Rajbhandari (2015) and Erwin *et al*. (2022) to analyse the flavonoid and phenolic antioxidant content in the test material. The antioxidant standard used was quercetin to test the total flavonoid content, so the total flavonoid test was equated with quercetin (Quercetin Equivalent = QE). Meanwhile, the total phenolic test used the gallic acid standard (Gallic Acid Equivalent = GAE). For comparison of flavonoid and phenolic contents, *A. capillaris* flower was used as a reference herbal extract.

For the flavonoid assay, a standard curve was prepared. A 10.0 mg quercetin standard solution was treated with 5% sodium nitrite (0.3 mL), followed by 10% aluminium chloride (0.6 mL). Two millilitres of 1 M sodium hydroxide were also added to the mixture after five minutes. Using a volumetric flask, the mixture was diluted with distilled water to a final volume of 10 mL. The mixture was transferred into a cuvette, maintaining the absorbance at 510 nm. To determine the sample's total flavonoid content, a 0.20 mL aliquot was added to 0.3 mL of 5% sodium nitrite. After 5 minutes, 10% aluminum chloride (0.6 mL) was added. After waiting for 5 more minutes, 1 M sodium hydroxide (2 mL) was also added. The mixture was diluted with distilled water as needed until a 10 mL volume was obtained in a volumetric flask. Lastly, the mixture was transferred to a cuvette, with absorbance measured at 510 nm (Pandey and Rajbhandari 2015; Erwin *et al*. 2022).

For the phenolic assay, a standard curve was also prepared. The 10.0 mg of gallic acid standard was added to Folin-Ciocalteu reagent (0.5 mL), and aquabidest (7.5 mL). After the mixture was left at room temperature for 10 minutes, 1.5 mL of 20% sodium carbonate was added. After 20 minutes of heating in a water bath at 40°C, the mixture was rapidly chilled in cold water. The mixture was diluted with aquabidest to a volume of 10 ml. The mixture was transferred to a cuvette, maintaining the absorbance at 760 nm. For the determination of total phenolic content in the sample, we carefully took 0.20 mL of the sample and added Folin-Ciocalteu reagent (0.5 mL) and distilled water (7.5 mL). The mixture was allowed to stand at room temperature for 10 minutes, then 20% sodium carbonate (1.5 mL) was added. Distilled water was added to bring the total to 10 mL to dilute the mixture as needed. We then transferred the mixture to a cuvette, maintaining the absorbance at 760 nm (Pandey and Rajbhandari 2015; Erwin *et al*. 2022).

2.4. Antioxidant Activity

The antioxidant activity assay was conducted based on a prior study by Phongpaichit *et al*. (2007) with slight modification using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. Briefly, ethanol extracts of secang wood (0.002; 0.004; 0.006; 0.008; 0.010 mg/mL), *A. capillaris* flower extract (0.009; 0.018; 0.027; 0.036; 0.045 mg/mL), and the control were dissolved in ethanol and made in various concentrations. Each was put into a test tube. Additionally, each test tube was filled with 1 mL of a 0.4 mM DPPH solution. The absorbance was measured at 517 nm after ethanol was added to a final volume of 5 mL, and the mixture was incubated at 37°C for 30 minutes. The regression equation was used to determine each 50% inhibitory concentration (IC_{50}) value. The regression equation was used to assess the IC_{50} value using SPSS version 26. For comparison of antioxidant activity, *A. capillaris* was used as a reference herbal extract (Phongpaichit *et al*. 2007).

2.5. Fibroblast Culture

The subjects of this study were human fibroblasts cultured in Dulbecco's Modified Eagle Medium (DMEM) and subjected to passage subculture 3-5. Fibroblast cultures came from human prepuce skin tissue obtained from circumcision. The inclusion criteria in this study

were dermis skin from the prepuce of healthy humans aged less than 12 years. The exclusion criterion in this study was infected prepuce skin.

The tissue obtained was then transferred from the transport medium (DMEM + Penstrep 2% + Fungizone 2%) and placed in a 6-well Petri dish containing 6 ml of FBS. The tissue was soaked in a 10% povidone-iodine solution for 3 minutes. Then, it was thoroughly washed with sterile phosphate-buffered saline (PBS) until the red colour of povidone-iodine was no longer visible. The layers of the epidermis and dermis were separated using sterile tweezers. The dermis tissue was then cut into pieces measuring 0.5 x 0.5 cm. The dermis tissue that had been cut into pieces was transferred into small flasks using Pasteur's tweezers. A 1 ml DMEM medium was added to the flask, then the flask was placed in the incubator at 37°C with 5% CO_2 for 24 hours. The DMEM medium was replaced daily for 3 days, or until the tissue was fully attached. Once the tissue was attached, the medium needed to cover the tissue thoroughly. The fibroblasts were ready for subculture when they covered 50% of the flask surface.

2.6. Blue Light Exposure on Fibroblasts

Cell viability and collagen synthesis assays in fibroblasts exposed to blue light were carried out using the BIO-Light® Brand LED Skin Rejuvenation tool, calibrated by the Indonesian National Standardisation Agency with 95% confidence. Based on previous studies, the dose that reduces fibroblast viability without toxic effects is 30 J/cm² (Park *et al*. 2022). In this study, to determine the optimal blue light dose for evaluating the effect of secang wood extract on cell viability after blue light exposure, we tested five different blue light doses using calibrated LED devices. Measurement results at a distance of 4 cm have an irradiation value of 54.72 watts/m² or 0.005472 watts/cm². Blue light was given in various doses and time exposure with group codes as follows: BL 13.7 J: 13.7 J/cm² (41 min 44 sec); BL 16.4 J: 16.4 J/cm² (49 min 56 sec); BL 21.6 J: 21.6 J/cm² (65 min 47 sec); BL 27.5 J: 27.5 J/cm² (83 min 46 sec), BL 32.8 J: 32.8 J/cm² (99 min 56 sec); and control: without any exposure.

2.7. Fibroblasts Viability Assay

For fibroblasts viability assay, according to a prior study by Park *et al*. (2022) and our pre-eliminary study, eight treatment groups using six different concentrations of *C. sappan* ethanol extract (7.8 µg/mL, 15.6 µg/mL, 31.3 µg/mL, 62.5 µg/mL, 125 µg/mL, 250 µg/mL) and a control group without any treatment, with optimum dose of blue light (21.6 J/cm² (65 minutes 47 seconds)), were chosen as shown in Table 1. The group codes in Table 1 were defined as follows: C: Control without treatment; BL: Blue light exposure without *C. sappan* extract; Cs1: *C. sappan* group 1; Cs2: *C. sappan* group 2; Cs3: *C. sappan* group 3; Cs4: *C. sappan* group 4; Cs5: *C. sappan* group 5; Cs6: *C. sappan* group 6. These research procedures were also carried out in three repetition processes (triplication). Fibroblasts' viability was assessed 24 hours after the blue light was applied to the cells. A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (100 µg/mL) was added to fibroblast cultures and incubated at 37°C for 3 hours. The result was formazan precipitation dissolved in dimethyl sulfoxide (DMSO). A spectrophotometer was used to measure absorbance at 542 nm. The absorbance values were used to assess fibroblast viability. Fibroblast viability was calculated by comparing the average optical density (OD) of the test

Table 1. Control and treatment groups in the viability assay and collagen synthesis assay

Viability assay								
Treatment	C	BL	Cs1	Cs2	Cs3	Cs4	Cs5	Cs6
Blue light exposure	None	21.6 J/cm² (65 m 47 s)						
C. sappan extract	None	None	7.8 µg/mL	15.6 µg/mL	31.3 µg/mL	62.5 µg/mL	125 µg/mL	250 µg/mL

Collagen synthesis assay						
Treatment	C	BL	Cs1	Cs2	Cs3	Cs4
Blue light exposure	None	27.5 J/cm² (83 m 46 s)				
C. sappan extract	None	None	7.8 µg/mL	15.6 µg/mL	31.3 µg/mL	62.5 µg/mL

C: Control without treatment; BL: Blue light exposure without *C. sappan* extract; Cs1: *C. sappan* group 1; Cs2: *C. sappan* group 2; Cs3: *C. sappan* group 3; Cs4: *C. sappan* group 4; Cs5: *C. sappan* group 5; Cs6: *C. sappan* group 6; m: minutes; s: seconds

groups with that of the control group. The OD value of the control was considered to be 100%. Fibroblast cell viability was measured at a single time point: 24 hours (Park *et al*. 2022).

2.8. Collagen Synthesis Assay

According to prior study by Park *et al*. (2022) and our pre-eliminary study, this assay involved six treatment groups using four different concentrations of secang wood (7.8 µg/mL, 15.6 µg/mL, 31.3 µg/mL, 62.5 µg/mL) ethanol extract and control without any treatment, with optimum dose of blue light (27.5 J/cm^2 (83 min 46 sec) as shown in Table 1. These research procedures were carried out in three repetition processes (triplication). The amount of collagen synthesised was measured in fibroblast cultures after blue-light exposure using the Sirius Red Collagen Assay colourimetric test. A spectrophotometer (570 nm) was used to measure optical density. Collagen synthesis can be calculated based on the absorbance results. Collagen synthesis was calculated as the ratio of the OD of each test group to that of the control group. The control OD value was considered to be 100%. Measurement of collagen synthesis was carried out 72 hours after blue-light irradiation and expressed as a percentage (Chen *et al*. 2013).

2.9. Statistical Analysis

This study presented the data analysis results as average values and displayed them in graphical form. The data analysis was performed using the SPSS program version 26. The normality and homogeneity test was conducted using the Shapiro-Wilk test. The difference in the average viability of cells in each treatment group compared to the control group was analysed using one-way ANOVA and the LSD post hoc test, since the data were normally distributed and homogeneous.

3. Results

3.1. Total Flavonoid and Phenolic Content

According to the total flavonoid content assay, the average flavonoid content was 7.41% (w/v) quercetin equivalents (QE), equivalent to 5.93 mg QE/100 mg in 5 mL of secang wood ethanol extract. While in the *A. capillaris* extract, 0.6%, there was 0.22% flavonoids equivalent to QE or 4.45 mg QE/100 mg. Therefore, secang wood ethanol extract had a higher total flavonoid content, as measured by QE, than *A. capillaris* (0.6%), the reference herbal extract.

According to the total phenolic test, an average of 3.57% gallic acid equivalents (GAE), or 2.86 mg GAE/100 mg, was found in 5 mL of secang wood ethanol extract. Meanwhile, 0.6% of *A. capillaris* extract contained 1.25% of the total phenolic equivalent gallic acid/GAE or 1.00 mg GAE/100 mg. Therefore, the ethanol extract of secang wood had a total phenolic content equivalent to gallic acid, which was slightly higher than that of *A. capillaris* (0.6%) as a reference herbal extract.

3.2. Antioxidant Activity

Measurement of antioxidant activity was performed by observing the capture of DPPH free radicals with the DPPH assay. In this test, the higher the sample concentration, the higher the percentage of free-radical capture. In the antioxidant test of secang wood ethanol extract, a concentration of 0.010 mg/mL captured a prominent radical of 64.023% (Table 2). Meanwhile, in the antioxidant test of *A. capillaris*. extract, a higher concentration than the secang wood ethanol extract was needed to capture prominent radicals (Table 2).

In the second wood ethanol extract, the IC_{50} value was observed at 0.0078 mg/mL, equivalent to 7.8 µg/mL (Figure 1). Based on the classification of antioxidant

Table 2. Antioxidant capacity of *C. sappan* wood ethanol extract and *A. capillaris* flower extract with DPPH assay

Concentration (mg/mL)	DPPH absorbance			%Free radical scavenging			Mean
	I	II	III	I	II	III	
C. sappan							
0.002	0.806	0.806	0.807	13.6	13.6	13.5	13.6
0.004	0.676	0.675	0.677	27.5	27.7	27.4	27.5
0.006	0.589	0.589	0.588	36.9	36.9	37.0	36.9
0.008	0.447	0.447	0.446	52.1	52.1	52.2	52.1
0.010	0.336	0.336	0.335	64.0	64.0	64.1	64.0
A. capillaris							
0.0090	0.738	0.739	0.740	14.0	13.9	13.8	13.9
0.0180	0.575	0.575	0.574	33.0	33.0	33.1	33.0
0.0270	0.420	0.419	0.418	51.0	51.2	51.3	51.2
0.0360	0.266	0.267	0.267	69.0	68.9	68.9	68.9
0.0450	0.167	0.167	0.166	80.5	80.5	80.7	80.6

activity, this concentration was classified as very strong (IC_{50} <50 µg/mL). In 0.6% *A. capillaris* extract, the IC_{50} was observed at 0.0273 mg/mL, equivalent to 27.3 µg/mL (Figure 1). Based on the classification of antioxidant activity, this concentration was classified as very strong (IC_{50} <50 µg/mL). Although both secang wood and *A. capillaris* extracts demonstrated very strong antioxidant activity, secang wood exhibited a comparatively greater potential. This was reflected in its lower IC_{50} value (IC_{50} 7.8 µg/mL) of secang wood, compared to A. capillaris (IC_{50} of 27.3 µg/mL), indicating that a smaller concentration of secang wood extract was required to achieve the same inhibitory effect.

3.3. Viability of Fibroblasts After Blue Light Exposure

Before exposure to blue light, the optimal testing dose was determined to reduce fibroblast viability. Based on the graph in Figure 2, the lowest dose of blue light irradiation that can significantly reduce fibroblast viability without causing toxic effects is 21.6 J/cm² or 65 min 47 sec. Each treatment group was tested for normality with the Shapiro-Wilk test. The test results showed that most groups had p-values greater than 0.05, indicating a normal distribution of the data. To assess the impact of secang wood ethanol extract on fibroblast vitality across various indices, a one-way ANOVA was used. The ANOVA statistical test aims to determine differences in the means of more than two sample groups. The ANOVA test can be performed if the data are normally distributed and homogeneous. Based on the normality and homogeneity test results, the significance values were greater than 0.05, so the data were declared normal and homogeneous, and parametric tests could be carried out. The test yielded a p-value < 0.05, indicating that the average viability of fibroblasts across groups was significantly different. The following shows fibroblast viability, expressed as optical density after exposure to blue light.

Based on the results of this study, a comparison was made between the control group and the treated groups exposed to blue light at different secang wood concentrations (Cs1-Cs6). A significant difference was observed in statistical analysis between control variables with blue light exposure and Cs1, Cs2, and Cs3 ($p<0.05$). In addition, negative mean-difference results indicate that Cs1, Cs2, and Cs3 have a very strong and significant effect on increasing the viability of fibroblasts exposed to blue light.

Controls with blue light exposure showed a significant decrease in viability compared with controls without blue light exposure. Ethanolic extract of secang wood at concentrations of 7.8 µg/mL, 15.6 µg/mL, and 31.3 µg/mL resulted in a significant, dose-dependent increase in viability after blue-light exposure. Moreover, the minimum dose of secang wood ethanol extract that showed significant recovery of fibroblast viability was 7.8 µg/mL (Figure 3).

3.4. Collagen Synthesis of Fibroblast Cultures Exposed to Blue Light and Given Various Concentrations of Secang Wood Ethanol Extract

In Figure 4, it can be seen that some groups that received blue light experienced a significant collagen synthesis decrease in comparison to fibroblast culture

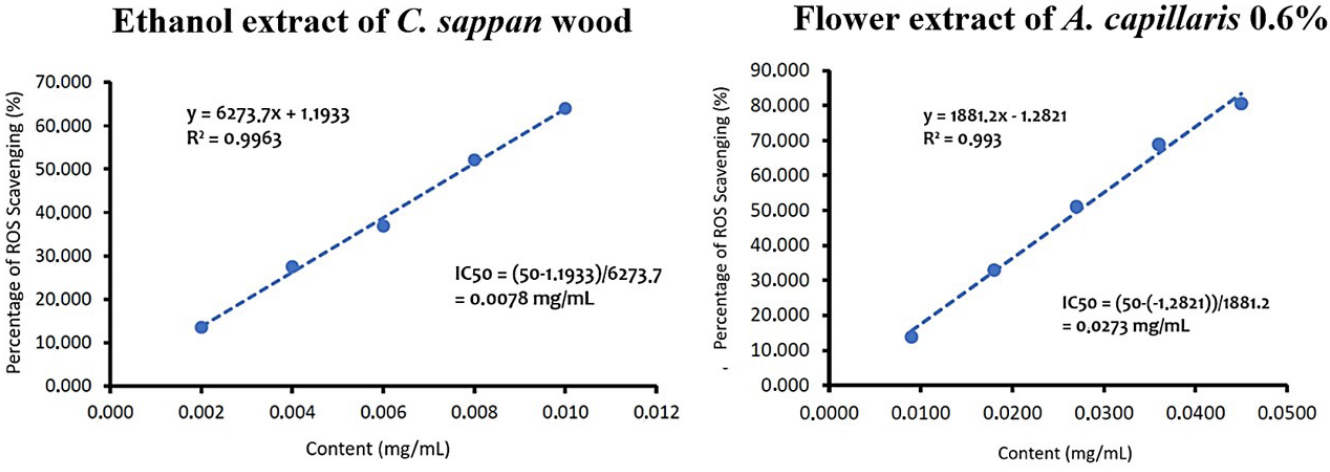

Figure 1. Regression curve of *C. sappan* and *A. capillaris* 0.6% with percentage of free radical scavenging to determine IC_{50}

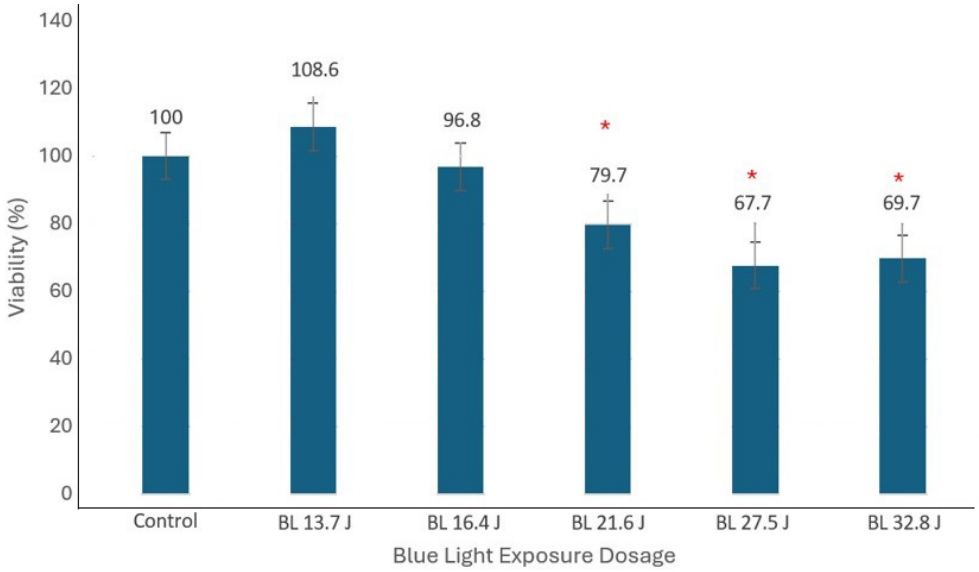

Figure 2. Blue light (BL) exposure dose-dependently decreased fibroblasts' viability (%). (*) significantly different ($p < 0.05$, LSD test) from the control group that was not exposed to blue light. Description: BL 13.7 J = blue light 13.7 J/cm^2, BL 16.4 J = blue light 16.4 J/cm^2, BL 21.6 J = blue light 21.6 J/cm^2, BL 27.5 J = blue light 27.5 J/cm^2, BL 32.8 J = blue light 32.8 J/cm^2, control = no blue light exposure

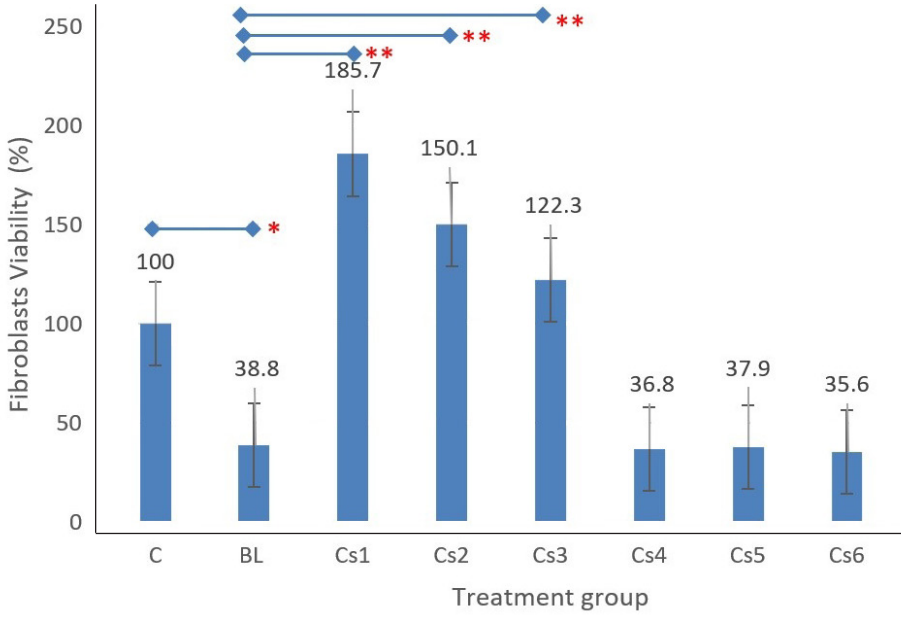

Figure 3. Fibroblasts' viability post-blue light (BL) exposure in various treatment groups. (*) Significantly different ($p<0.05$) from the control group that was not exposed to blue light; significantly different ($p < 0.05$, LSD test) from the BL group. Description: C: fibroblast culture without *C. sappan* extract and without blue light exposure; BL: fibroblast culture without *C. sappan* extract exposed to blue light for 65 minutes 47 seconds (21.6 J/cm^2); Cs1 = fibroblast culture with *C. sappan* extract 7.8 µg/mL exposed to blue light for 65 minutes 47 seconds (21.6 J/cm^2); Cs2 = fibroblast culture with of *C. sappan* extract 15.6 µg/mL exposed to blue light (21.6 J/cm^2); Cs3 = fibroblast culture with *C. sappan* extract 31.3 µg/mL exposed to blue light (21.6 J/cm^2); Cs4 = fibroblast culture with *C. sappan* extract 62.5 µg/mL exposed to blue light (21.6 J/cm^2); Cs5 = fibroblast culture with *C. sappan* extract 125 µg/mL exposed to blue light (21.6 J/cm^2); Cs6 = fibroblast culture with *C. sappan* extract 250 µg/mL exposed to blue light (21.6 J/cm^2)

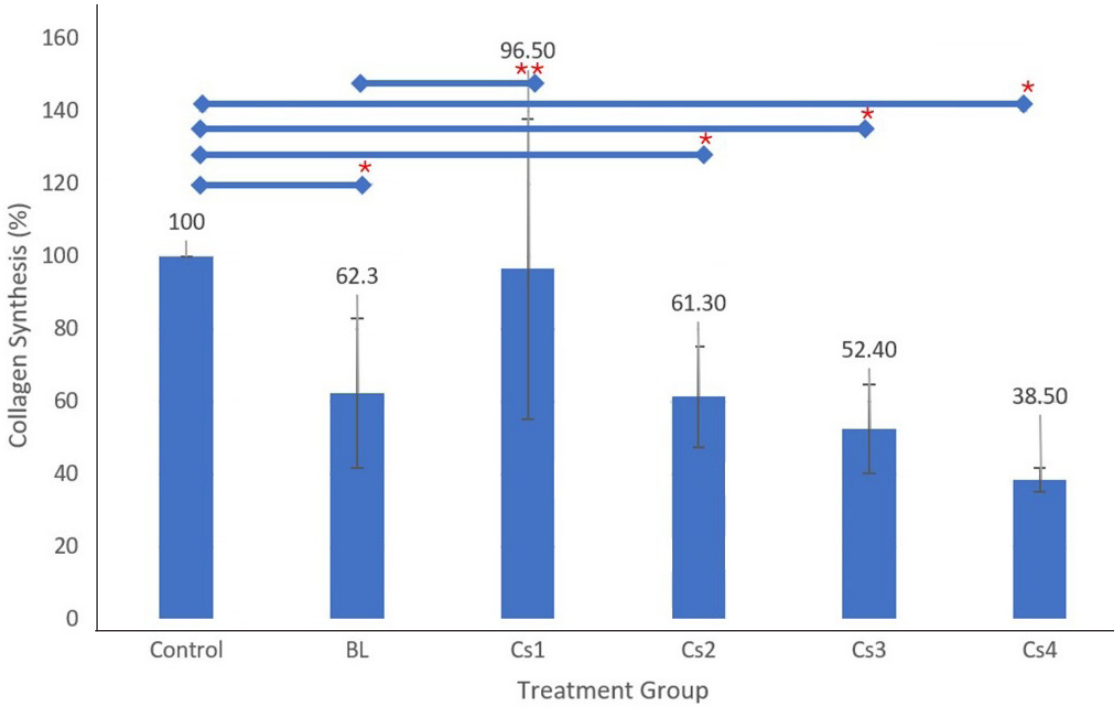

Figure 4. Collagen synthesis after 72 hours in fibroblast cultures exposed to blue light (BL) and receiving various concentrations of *C. sappan* (Cs). (*): significant ($p<0.05$, LSD test) in comparison to the C group; (**): significant ($p<0.05$) in comparison to the BL group. Description: C: fibroblast culture without *C. sappan* extract and without blue light exposure; BL: fibroblast culture without *C. sappan* extract exposed to blue light for 83 minutes 46 seconds (27.5 J/cm^2); Cs1: fibroblast culture with *C. sappan* extract 7.8 µg/ml exposed to blue light (27.5 J/cm^2); Cs2: fibroblast culture with *C. sappan* extract 15.6 µg/ml exposed to blue light (27.5 J/cm^2); Cs3: fibroblast culture with *C. sappan* extract 31.3 µg/ml exposed to blue light (27.5 J/cm^2); Cs4: fibroblast culture with *C. sappan* extract 62.5 µg/ml exposed to blue light (27.5 J/cm^2)

group without secang wood ethanol extract and without blue light exposure, namely the fibroblast culture group without secang wood ethanol extract (37.7% decrease; $p = 0.015$), fibroblast culture with secang wood ethanol extract concentration of 62.5 µg/mL (61.5% decrease; $p < 0.05$), 31.3 µg/mL (47.6% decrease; $p < 0.05$), and 15.6 µg/mL (38.7% decrease; $p < 0.05$). The fibroblast culture group with secang wood ethanol extract at a concentration of 7.8 µg/mL also showed a 3.5% decrease in collagen synthesis, but it was not significant ($p > 0.05$). Some groups of fibroblast cultures that received secang wood ethanol extract and blue light exposure experienced a decrease in collagen synthesis that was not significant compared to the group of fibroblast cultures without secang wood ethanol extract that were exposed to blue light. These groups were fibroblast cultures with secang wood ethanol extract concentrations of 62.5 µg/ml (23.8% decrease; $p>0.05$), 31.3 µg/ml (9.9% decrease; $p>0.05$), and 15.6 µg/ml (1% decrease; $p>0.05$). The group of fibroblast cultures with secang wood ethanol extract concentrations of 7.8 µg/ml (Cs1) experienced a significant increase in collagen synthesis of 34.2% ($p<0.05$) compared to fibroblasts exposed to blue light without secang wood ethanol extract (BL group).

4. Discussion

Our study performed phytochemical analysis, antioxidant activity, cell viability, and collagen synthesis assays on fibroblasts exposed to blue light. For the phytochemical analysis, we focused only on flavonoids and phenolics because these compounds are the major components with antioxidant activity (Huyut *et al.* 2017). Furthermore, *A. capillaris* was used as a reference herbal extract for phytochemical and antioxidant activity analysis and was not included in the cell viability and collagen synthesis assays.

The results of our study show a higher quercetin content in secang wood ethanol extract (7.41%) than previous research, which reported a flavonoid content of 6.02% in secang wood ethanol extract (Nomer *et al.* 2019). These slightly different results are likely due to the origin of the test material and different extraction methods. Casagrande *et al.* (2006) stated that topical formulations

containing the flavonoid quercetin are effective, owing to their antioxidant properties in reducing UVB-induced oxidative stress and inflammatory responses (Casagrande *et al*. 2006). Thus, secang wood ethanol extract, which has a high content of flavonoids equivalent to quercetin, has the potential to be developed as a topical formulation for the protection/prevention of skin damage against sun exposure, including ultraviolet rays and blue light.

The ethanol extract of secang wood in this study has a total flavonoid content of 5.93 mg QE/100 g, higher than the reference herbal extract, *A. capillaris* extract, at 0.6% (3 mg QE/100 g). In addition, when we compared to another common fruits, the flavonoid content of secang wood ethanol extract also higher from various common plants products, such as, Fuji apple (2.35 mg QE/100 g), guava (1.00 mg QE/100 g), black grapes (2.00 mg QE/100 g), and banana (0.06 mg QE/100 g) (Haytowitz *et al*. 2018).

The secang wood ethanol extract in our study also has a total phenolic content of 2.86 mg GAE/100 g, higher than the reference herbal extract, *A. capillaris* extract, at 0.6% (1.00 mg GAE/100 g). In addition, when we compared to another common fruits, the phenolic content of secang wood ethanol extract also higher from various common plants products, such as, avocado (1.2 mg GAE/100 g), longan (1.6 mg GAE/100 g), mango (2.4 mg GAE/100 g), and jackfruit (0.9 mg QE/100 g) (Soong and Barlow 2004).

Flavonoid and phenolic compounds in food have antioxidant properties. Both of these chemicals have the capacity to scavenge and prevent free radicals. This forms the basis for why flavonoids and phenolics are used for their antioxidant potency (Huyut *et al*. 2017; Pietta 2000).

Quercetin and phenolic compounds act as antioxidants because they have hydroxyl groups that can release H^+. The difference in solvent polarity determines the chemical structure of the extracted phenolic compounds. Phenolic and flavonoid compounds act as antioxidants by blocking prooxidant enzymes, boosting antioxidant enzymes, or combating free radicals (Hardinsyah *et al*. 2019). A reliable and popular method for determining the overall antioxidant capacity of natural extracts is the DPPH radical-scavenging assay. The IC_{50} values are frequently used to report assay results (Phongpaichit *et al*. 2007).

The IC_{50} value means that the compound concentration is able to reduce 50% of the activity of free radicals, in this case, DPPH. According to Phongpaichit *et al.* (2007), if a compound's IC_{50} is less than 50 µg/mL (or 50 ppm), it is considered a very powerful antioxidant; if it is between 50 and 100 µg/mL, it is considered strong; if it is between 101 and 150 µg/mL, it is considered moderate; and if it is between 151 and 200 µg/mL, it is considered weak. A prior study by Park *et al.* (2022) showed that secang wood ethanol extract has dose-dependent DPPH antioxidant activity. This study did not calculate the IC_{50}, but from the results obtained, it was estimated that the IC_{50} was at a value of around 62.5 µg/mL. The different result was shown in our study, that the IC_{50} from flavonoid and phenolic compounds in the food are acknowledged for having antioxidant qualities ethanol extract is 7.8 µg/mL. There were differences in extraction methods (sample origin, solvent concentration, preparation, and processing techniques) between our study and a study by Park *et al*. (2022). Research by Park *et al*. (2022) used crushed heartwood particles (<50 mesh) extracted with 80% ethanol (1:10, w/v) directly for one day, followed by filtration with Whatman paper and additional microfiltration through a 0.22-µm PVDF membrane to ensure purity before concentration and drying using a rotary evaporator under low pressure at 50°C. In contrast, our study specifically reported the sample origin (Bogor, West Java), involved additional preparation steps such as sorting, washing, and drying the heartwood before grinding it into fine powder, and macerated three times (24 hours each) at room temperature using 96% ethanol. Moreover, our study used a simpler method using conventional filtration and rotary evaporation for concentration without microfiltration or low-pressure drying. On the other hand, 0.6% *A. capillaris* extract showed a higher IC_{50} value of 27.3 µg/mL. This could be connected to the greater flavonoid and phenolic content in the ethanol extract of secang wood compared to *A. capillaris* in our study.

The value of IC_{50} in the DPPH scavenging process showed that flavonoid and phenolic compounds play a major role in its antioxidant activity (Fitriansyah *et al*. 2018). After knowing the antioxidant potential, our research continued by testing the extract's biological activity to observe its ability to prevent damage, which was measured through cell viability after exposure to blue light.

Our study revealed that blue light significantly reduced fibroblast viability, with the minimum light dose being 21.6 J/cm^2 over 65 min 47 sec. However, the specific blue-light tool used can yield varying outcomes. According to Park *et al*. 2022 that blue light with 400-440 nm wavelength and a power of 30 J within 1 hour and 7 minutes can increase oxidative stress and cytotoxicity.

This finding is also supported by Ge *et al.* (2023), who showed that exposure to blue light with a dosage of ≤ 6.5 J/cm^2 revealed no impact on cells, whereas doses ≥ 26 J/cm^2 resulted in cytotoxicity for fibroblasts. Therefore, a dosage range of 6.5-26 J/cm^2 is considered to have a biological effect without causing cytotoxicity (Ge *et al*. 2023). High content of flavonoids and their antioxidant properties in secang wood may contribute to preventing damage in fibroblasts due to blue light exposure. These compounds interrupt the oxidation chain reaction, thus preventing free radicals from forming (Sucita *et al.* 2019; Hafshah *et al.* 2022).

The primary cells in the dermis responsible for the production and deposition of extracellular matrix proteins, such as collagen, are fibroblasts (Sadowska *et al*. 2021). Blue light can damage collagen and elastin, either directly from blue light exposure or indirectly through the production of ROS. Blue light can also induce matrix metalloproteinases (MMP) enzymes in cells, resulting in collagen degradation and inhibition of new collagen synthesis (Coats *et al*. 2021). Park *et al*. (2022) reported that exposure to blue light on fibroblasts at a dose of 30 J/cm^2 for 1 hour 7 minutes can reduce collagen type 1 and type 3, and increase MMP-1 and MMP-3. In our study, the decrease in collagen synthesis in fibroblast cultures exposed to blue light was statistically significant ($p<0.05$) and is in accordance with the results of other studies, which state that exposure to blue light can reduce collagen synthesis (Park *et al*. 2022).

In our study, fibroblast cultures treated with secang wood extract and exposed to blue light showed decreased collagen synthesis compared with control fibroblasts without extract and without blue light (Figure 4). Furthermore, fibroblasts given secang wood extract (7.8 µg/mL) and exposed to blue light also showed a non-significant decrease in collagen synthesis compared to controls without extract and without blue light ($p>0.05$). These findings showed that secang wood at a concentration of 7.8 µg/mL provides a protective effect against blue light-induced inhibition of collagen synthesis. However, secang wood extract at concentrations of 62.5 µg/mL ($p<0.05$), 31.3 µg/mL ($p < 0.05$), and 15.6 µg/mL ($p < 0.05$) significantly reduced collagen synthesis in fibroblasts. This indicates that while lower concentrations of secang wood extract (7.8 µg/ml) may have a protective effect against blue light–induced suppression of collagen synthesis, higher concentrations can be detrimental by significantly impairing collagen production in fibroblasts, possibly due to cytotoxic or pro-oxidant effects at elevated doses (e.g., brazilin component of secang wood showed higher cytotoxicity to fibroblasts) (Puttipan *et al*. 2018; Asevedo *et al*. 2025) and may synergize with the effect of blue light exposure to increase ROS, activate MMP-1 and inhibit collagen gene expression in human fibroblasts (Ge *et al*. 2023). Due to the potential synergistic cytotoxic effects of this combination, careful dosage optimisation is essential in cosmeceutical applications (Puttipan *et al*. 2018).

Our research has not been directly directed at developing cosmetic products, but rather focused on exploring Indonesian natural materials as potential cosmetic ingredients to prevent blue light–induced skin damage. Based on our study, the ethanol extract of secang wood from Bogor, West Java, Indonesia, has potential as a cosmetic ingredient to protect skin from damage caused by visible light, particularly blue light (Morvan *et al*. 2019; Park *et al*. 2022). These results form the basis for further in vivo research to evaluate skin protection from blue light.

In conclusion, secang wood ethanol extract has high levels of total flavonoids and phenolics. Moreover, it showed antioxidant activity and significantly inhibited the decrease in fibroblast viability and collagen synthesis after blue light exposure.

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